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7.

Mechanisms of Neural Maturation

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The development of behavior is directly dependent upon an orderly assembling of the neurons of the nervous system into appropriate patterns of interconnections. Although neural maturation involves certain other factors, such as the adjustment of excitation thresholds, the present chapter is concerned almost entirely with what appears to constitute its principal and most problematic feature, namely, the developmental organization of those elaborate and intricate patterns of neuronal linkages necessary to adaptive function. Knowledge of this phase of neurogenesis is at present still little beyond the theoretical stage, and it extends only to relatively simple aspects of the integrative structure, such as the connections between center and periphery and the central synaptic associations of the more direct reflex circuits. However, study of the developmental organization of these elementary integrative relations may be expected to yield general principles of maturation applicable as well to the more complex behavior patterns.

When the problem is reduced for the purpose of analysis into its most simplified form, it may be stated as follows: *how do the outgrowing axons and dendrites of the developing nerve cells manage to form and to maintain proper end-organ terminations in the periphery and proper synaptic associations within the centers?* The problem requires particular regard to the differential specificity with which the neuronal connections are established. It demands answers, for example, to such questions as the following: How do motoneurons innervating a flexor muscle acquire central reflex relations different from those acquired by neighboring motoneurons innervating an extensor muscle? How are optic fibers from different retinal areas able to establish their synaptic relations in the visual centers so that the retinal field becomes projected onto the brain in an orderly manner, permitting objects in space to be seen discretely and localized accurately? Why, again, should the sensory fibers of the horizontal semicircular canal of the labyrinth, on growing into the medulla, form reflex associations different from those formed by accompanying sensory fibers entering the same vestibular centers by the same route, but associated peripherally with the vertical canals? How are the secondary central neurons, which link the different types of sensory fibers with the various motor systems, enabled to form their intracentral associations in the particular patterns necessary for adaptive reflex function? In the course of growth, sensori-neuromotor associations are somehow laid down in a consistent, orderly manner. How are such patterns organized in development? What is the nature of the regulative processes involved?

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"Maturation" versus Learning

A first problem to be considered is the extent to which the neuronal associations are (1) preformed directly by processes of growth and cell differentiation and (2) patterned by functional regulation through experience and training. Where adaptation by function is indicated, we are led into the problems of the physiological basis of conditioned reflexes and learning. On the other hand, where inherent predetermination is indicated, further analysis must deal with the embryological, cytological, and physicochemical phenomena involved in the growth, migration, and differentiation of nerve cells, and in the formation of contact relations with other neurons and with their respective end organs.

The maturation-learning problem has long been a subject of controversy. In 1931 Holt, among others, was arguing that none of the patterning of synaptic connections between sensory, central, and motor neurons is inherently predetermined. He maintained that developmental forces leave the nervous system an unorganized, equipotential network capable of nothing but diffuse, random reaction. Out of initially random movements, neural organization is gradually achieved, according to this view, through the supposed neurobiotactic outgrowth of dendrites toward axons excited in the course of activity. The most primitive reflexes and even the primary synaptic connections of the peripheral nerves, as well as all higher integrative relations, were presumed to be patterned functionally in this manner.

Since the proposal of this extreme hypothesis, however, contradictory data have been accumulating until now the pendulum of opinion is swinging sharply in the opposite direction toward increasing recognition of the forces of inheritance (Hunter, 1947). A survey of the evidence as it now stands leads to the conclusion that the basic patterns of synaptic association throughout the vertebrate nervous system are organized for the most part by intrinsic forces of development without the aid of learning. This would include most of the integrative structure of the spinal cord and brain stem. At higher levels of the brain, even in the primates, all the intricate interrelations known to neuroanatomy, such as the various projection systems to and from the cerebral and cerebellar cortices and the interconnecting systems between cortical areas — in so far as they are constant within a species — must likewise be included among the relations of the nervous apparatus subject to inherent organization. By process of elimination the interneuronal relations patterned by learning would seem to be relegated to those circuits farthest removed from the direct conduction pathways which, in the mammal, would be confined mainly to the cerebral cortex. Regarding the limits and overlap of the respective influences of learning and maturation, the evidence remains vague and incomplete.

For present purposes it is sufficient to recognize that the basic integrative architecture of the nervous system is organized directly in the growth process itself. Although the building of integrative circuits presents a fascinating embryological problem in its own right, it is considered here primarily with regard to its implications for the structure and function of the adult nervous system. Knowledge of how inherent behavior is installed may give some clues to the neural basis of learned behavior. The nature of central nervous integration and of the structural framework subserving it should become clearer in the light of such information.

GROSS MORPHOGENESIS

The precise patterning of neuronal interconnections constitutes the ultimate and most refined step in the development of the nervous system. A proper final adjustment of the synaptic associations, however, is dependent upon preceding grosser phases of development in which are attained the gen-

eral form, size, and location of the major parts of the nervous system.

The manner in which the embryonic neural tube becomes transformed into the adult brain and spinal cord is depicted in many standard texts. The histological picture has also been described in considerable detail, particularly for the earlier stages of development, including the proliferation, polarization, differentiation, and migration of the neuroblasts, the outgrowth of the axons and dendrites, the appearance and enlargement of the various nuclear fields, the laying down in successive steps of many of the main fiber tracts, and their progressive enlargement and myelinization (see papers and references of Barron, Coghill, Herrick, Langworthy, and Windle).

At first the nerve fibers are few in number, span very short distances, and interconnect only a few nuclear masses. In the course of growth many more fibers are added to the original "pathfinder" lines, and the short fibers become lengthened and drawn into circuitous courses. Additional nuclear fields appear, and new connecting links are laid down to form a structure of ever-increasing complexity. There are successive shifts in topographical relations and a nicely geared timing of the developmental events.

Further insight into these phases of development has been added by the methods of experimental embryology, which have begun to unravel some of the regulative forces involved (see reviews of Detwiler, 1936; Harrison, 1935; Herrick, 1925, 1933, 1948; Piatt, 1948; Weiss, 1939, 1941c). It has been found, for example, that fibers of the sensory nerve roots, on entering the brain, tend to be deflected into those nuclear centers that happen, at the time, to be undergoing cellular proliferation. Similarly, outgrowing fibers of the peripheral nerves verge toward rapidly growing peripheral organs. Likewise intracentral fiber tracts tend to form connections with those nuclei undergoing accelerated proliferation at the time of the fibers' outgrowth. As successive constellations of proliferative foci make their appearance, new patterns of connecting links are established. In all these instances the influence of proliferative centers on the direction of nerve outgrowth appears to depend upon the formation of lines of force in the interstitial medium which converge on the given growth centers and which in turn cause a converging alignment of the ultramicroscopic particles along which the tips of the nerve fibers are mechanically guided (Weiss, 1941c). That specific chemical factors may also be involved is suggested in the studies of Hooker (1930) who rotated a piece of the spinal cord and found a tendency for the fiber bundles to reconnect with their corresponding fascicles in the rotated segment.

The ingrowth of fibers into a given nuclear field tends to stimulate the growth of the invaded center and to enhance the development of its dendritic ramifications. A correlation has been demonstrated between the size of peripheral structures and the quantitative development of their associated spinal ganglia and central nuclei. The ultimate basis of such effects is still in doubt, but the evidence (Barron, 1945; Hamburger, 1946; Levi and Levi, 1942) suggests that these phenomena are correlated entirely with the growth phase of the nerve and end-organ tissues, and not with their functional phase.

The gross architecture of the nervous system, including the locations of nuclear centers and the patterns of fiber tracts, is the result of a manifold interaction of numerous growth factors. Mechanical and spatial relations, and the mechanisms of cell proliferation, migration, and differentiation, along with embryonic induction, are all involved. The whole process depends upon a neat timing of the developmental steps. Clearly this grosser patterning is determined independently of activity, in a manner characteristic of the species. Even individual variations, almost as characteristic of brain structure as of the face (Lashley, 1947), seem to be genetically determined.

REFINED PATTERNING OF INTEGRATIVE CIRCUITS

It is not enough, of course, that fiber tracts terminate in particular nuclei. The fibers of each tract must, in addition, form the proper synapses within each nucleus of termination. For example, the optic tract in the amphibian must not only make its connection with the optic tectum instead of with other regions of the brain, but the separate fibers composing the tract must terminate in a selective, individuated fashion within the optic tectum itself. The sensory fibers of each spinal nerve must likewise form associations appropriate to the modality of their end organs and to the particular ligament, tendon, muscle, joint, or region of skin, periosteum, or fascia, etc., in which the end organs are located. And within most nuclei localized circuits must be formed. These refined aspects of neurogenesis hold most interest from the functional point of view. It is the most difficult feature to analyze, and until lately our only explanatory concepts, such as "neurobiotaxis," "chemotaxis," "electrodynamic fields," etc., have been vague and speculative.

During recent years a series of experiments dealing with the establishment of synaptic associations in the amphibian central nervous system has given rise to a more definite "chemoaffinity" theory. This scheme attempts to account for the development of neural patterns in terms of cell differentiation and embryonic "induction" processes closely akin to those known to be involved in other organ systems. This aspect of neurogenesis has thereby been brought into line with better-understood phases of development, the main difference being that in the nervous system the final product is far more specific in design and more highly differentiated.

Because it is often easier to study the secondary growth of neuronal connections than to work with the initial embryonic growth, much of the available data is derived from experiments on nerve regeneration. All evidence indicates that the patterning in regeneration is regulated by essentially the same forces as in development.

End-Organ Connections

In the periphery, where the specific termination of neurons is open to observation, the problem of linkages is more accessible to experimental analysis than it is when central connections are involved. It is not usually maintained, even in theory, that learning influences the formation of end-organ connections. Indeed the peripheral relations are generally laid down well before the onset of reflex function. The fact that a precise patterning can be achieved in the periphery without the aid of learning lends credence to the idea that associations within the centers may likewise be adjusted by the forces of growth alone.

Electrical, mechanical, and chemical influences have each been thought at various times to play the predominant role in guiding nerve fibers to their destinations. Except for a slight directive effect of strong currents under special conditions of tissue culture (Marsh and Beams, 1946), the electrical theories have found little experimental support. On the other hand, the universal importance of mechanical factors has been clearly demonstrated. It was noted by Harrison (1914) that nerve fibers grow only in contact with a supporting surface, never in a homogeneous fluid medium. The later studies of Harrison (1935) and particularly of Weiss (1941*c*) have further emphasized the profound influence of the mechanical substrate on the guidance of growing nerve fibers. Even the ultramicroscopic particles of the interstitial fluid and the most delicate of interfacial films, as well as all larger structures, serve to deflect and to channel the fine filamentous pseudopodia of the advancing fiber tips.

Many aspects of the growth and termination of nerve fibers, however, cannot be accounted for purely in terms of contact

guidance. This is particularly true of the patterning of refined synaptic connections in the centers. From here on it will be taken for granted that mechanical factors are ubiquitous in nervous development and our attention will be concentrated on certain additional factors of more selective action which seem to be largely chemical in nature.

From observations of nerve development and regeneration it has been inferred (Cajal, 1928, 1929; Harrison, 1910; Tello, 1923; and others) that the different end organs must possess some kind of selective chemical affinity for the various types of outgrowing nerve fibers. In the development of the tongue, for example, the fibers of nerve XII establish connections selectively with the striated muscle cells; those of VII and IX connect with the cells of the taste endings; those of V form touch, pain, and thermal endings; and those of the autonomic system connect with the smooth muscle cells of the blood vessels. This selective termination seems to call for differential responses on the part of the outgrowing nerve fibers to specific chemical properties of the end organs. It need not be assumed that the advancing fiber tips are attracted from any great distance by chemicals diffusing from their prospective terminations. Presumably the interstitial pathways to terminal tissues are chemically conditioned by the types of cells surrounding them, and different nerve fiber types are prone to grow preferentially along certain of these diversely flavored pathways. This is suggested in the predisposition of sensory and motor fibers to form separate distal branches in the developing limb bud. (Hamburger, 1929; Taylor, 1944).

Piatt (1942) contributed further evidence that the role played by peripheral nerve fibers in attaining their goal is not so passive and mechanically controlled as had previously been thought. Limbs reared on parabiotic salamander twins to an advanced stage of development in complete absence of innervation were grafted in place of the corresponding limb on animals otherwise normal. The resultant nerve pattern formed by the invasion of the host nerves into the developed "aneurogenic" limbs proved to be remarkably normal. Many features of the process, such as the penetration of cartilage masses by the nerves, the tunneling of the ulnar nerve through the belly of the ulnocarpal muscle instead of coursing around it, and the entrance of nerves into their muscles at the customary motor points, suggests a significant influence of chemical factors. The course of some of the cranial nerves is even more difficult to account for unless we assume chemical influences.

Once the fibers have reached their general region of termination, local chemical effects supposedly come into play to determine the specific type of end-organ cells with which the fibers will make connection (Cajal, 1929). In regenerating nerves in the tadpole tail, Speidel (1946) found that aberrant lateral-line fibers show a marked tendency to arrive at and innervate displaced lateral-line organ tissue, whereas no such tendency is ever displayed by spinal nerve sprouts nearby. Nerve regeneration studies, in general, reveal considerable latitude in the extent to which nerve fibers can be forced to form atypical terminations. On the other hand, some examples of strict incompatibility have been observed such as the inability of adrenergic fibers to form functional connections with cholinergic endings, and vice versa (Langley and Anderson, 1904), and the inability of sensory fibers to form transmissive junctions with muscle fibers (Langley and Anderson, 1904; Weiss and Edds, 1945).

Sometimes, instead of growing to their appropriate end organs, the nerve fibers may themselves induce the formation of the appropriate endings from indifferent tissue. In the catfish, for example, the taste buds as well as lateral-line organs are induced to develop at the tips of invading nerve fibers (Bailey, 1937; Olivo, 1928; Olmsted, 1931). On the other hand, the nerve fibers of the

general cutaneous system pervade the integument in the same regions, but these do not induce formation either of taste buds or of lateral-line organs (cf. also Speidel, 1948). Thus, within a given area of the skin, fibers from the gustatory nerve induce gustatory endings, fibers from the lateral-line nerve induce lateral-line endings (these two types of receptors being quite different histologically), but fibers from the general cutaneous nerves terminate freely without inducing any specialized endings.

The type of end organ that is formed may also be conditioned by the type of tissue into which the nerve grows (Bailey, 1937; Dijkstra, 1933). Still another type of terminal relation, discussed more fully below, has been found (Weiss, 1936) where the outgrowing nerves terminate indifferently on their end organs, after which the end organs induce biochemical specificity in the nerve fibers.

The early idea that peripheral nerve fibers differ from each other in their chemical make-up (Cajal, 1928; Hering, 1913; Langley, 1898) has thus been confirmed in studies of peripheral innervation. Neuron specificity influences end-organ connections in different ways in different regions. Nevertheless it is only one factor operating in conjunction with many others. Since connections in the periphery must be made with reference to the central connections, it is difficult to deal with the one apart from the other. Accordingly more detailed discussion of neuron specificity is postponed until it can be taken up in relation to central patterning.

Synapses of Sympathetic Ganglia

A relatively simple type of synaptic relation exists within the sympathetic ganglia of the autonomic system located outside the neuraxis. Preganglionic fibers emerge from the spinal cord via the ventral roots and make synaptic terminations upon the neurons of the sympathetic ganglia. These neurons in turn send their axons to visceral end organs. The neurons within a single ganglion may innervate a variety of end organs which function separately and which therefore require separate control from the centers. If the preganglionic fibers were to establish their synapses in a haphazard manner within a ganglion, excitation from the centers could lead only to massive, undifferentiated response. Excluding the possibility of specific nerve energies (see p. 272), selective activation must depend upon orderly synapsis within the ganglia, which, like the end-organ linkages, can hardly be ascribed to learning.

The early studies of Langley (1898, 1900) on nerve regeneration in the sympathetic system of the cat suggest that the synaptic patterning is determined by biochemical differences among the classes of neurons involved. The superior cervical ganglion in the cat innervates the smooth muscles of the auricular blood vessels, the nictitating membrane, the eyelids, the iris, the facial hairs, and the salivary glands. The preganglionic fibers reach the ganglion through the main sympathetic trunk, which they join after emerging from the spinal cord through different thoracic nerves from T1 to T7. Preganglionic fibers associated with different classes of ganglionic neurons tend to exit from the cord at different segmental levels.

Following severance of the sympathetic trunk, the preganglionic fibers regenerate to reestablish connections within the superior cervical ganglion. Despite the mix-up and disarrangement of the original fiber pattern in the nerve scar, inevitable under the conditions of the experiments, Langley found a remarkable tendency for the preganglionic fibers to restore their original type of linkages among the various classes of ganglionic neurons. After regeneration separate stimulation of the preganglionic fibers of the different thoracic nerves, T1–T7, evoked their typical selective action in the periphery. Langley stated that he could see no feasible means, except chemiotaxis, by which the preganglionic fibers could pick and choose

the particular ganglionic neurons with which they become associated. Hypoglossal fibers will also regenerate readily into the superior cervical ganglion and arborize profusely among the cell bodies, but unlike the preganglionic fibers they fail to form the proper synaptic structures necessary for impulse transmission (Hillarp, 1946).

As pointed out by Langley, chemical specificity of neurons may in many instances have only a preferential rather than an all-or-none effect on termination. In regeneration, for example, a given neuron may terminate more readily on the class of cells with which it was originally connected, but when the fibers cannot reach their favored terminals atypical connections may be formed. It is well substantiated that nerves can be forced by surgical measures, such as nerve crossing, to form atypical linkages under a wide variety of conditions. The readiness with which different types of neurons form terminal endings on other cells appears to vary from complete indifference to highly exclusive selectivity.

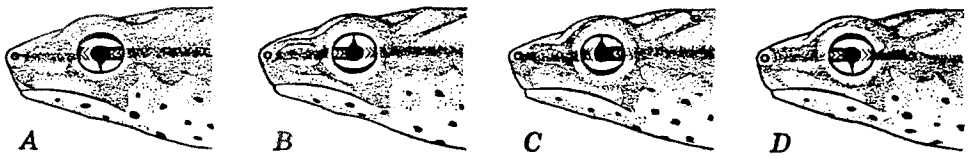

FIG. 1. Surgical rotation and inversion of the eye. It is possible to invert the eye completely by rotating it through 180 degrees, or the eye can be inverted on any one axis by transplanting it to the contralateral orbit. *A:* Normal orientation. *B:* 180-degree rotation. *C:* Dorsoventral inversion. *D:* Nasotemporal inversion.

Central Synapses of the Retina

Much of the experimental work on the patterning of central synapses has been performed on amphibians, partly because of the oft-cited advantages of this group for embryological study and partly because the amphibian central nervous system in larval stages, and even in the adult, retains a capacity for regeneration far greater than that of higher vertebrates. In amphibians, furthermore, adjustment by learning is not so much a complicating factor.

The functional relations between retina and brain centers must be patterned with orderly precision for visual perception. Each retinal locus must possess its unique "spatial sign." Anatomically it has been shown that the retinal quadrants have an orderly projection upon the brain centers throughout the vertebrate series from fish to man (Stroer, 1939). The problem therefore is to account for the selectivity with which the ingrowing optic fibers form their central associations.

It was shown by Matthey (1926*a*, *b*, *c*) that the optic nerve of the grafted adult urodele eye is capable of reestablishing functional connections in the brain. It was already known that most of the retina of such eyes disintegrates and is then regenerated from the surviving ciliary margin. In his microscopic examinations Matthey also observed an atypical meandering and intermixing of fibers in the regenerated optic nerves. Nevertheless his descriptions of the recovered visual reactions indicated accurate spatial localization and movement perception. Similar findings were later reported from Stone's laboratory (Stone and Ussher, 1927; Beers, 1929; Stone and Cole, 1943). Inclusion, among these earlier reports, of good visual recoveries following 90- and 180-degree rotation of the eye and following atypical connection of the optic fibers with the wrong brain center suggested that some sort of functional adaptation might be involved. Further analysis, as outlined below, however, contradicts this possibility in favor of organizational forces, strictly developmental in character.

It is possible in amphibians to rotate the eyeball on its optic axis through 180 degrees, leaving the optic nerve intact. The eye heals in this new position, with the dorsal quadrant of the retina placed ventrally in the orbit and with the temporal quadrant turned to the nasal side (see Fig. 1*B*). The visual responses then become reversed (Sperry, 1942*a*, 1943*b*). For example, the optokinetic reactions to movement of the visual field around the dorsoventral and rostrocaudal axes of the body are made in the direction opposite from normal, as illustrated in Fig. 2. The predatory reactions involving approach, pursuit, and striking at small moving objects are directed toward

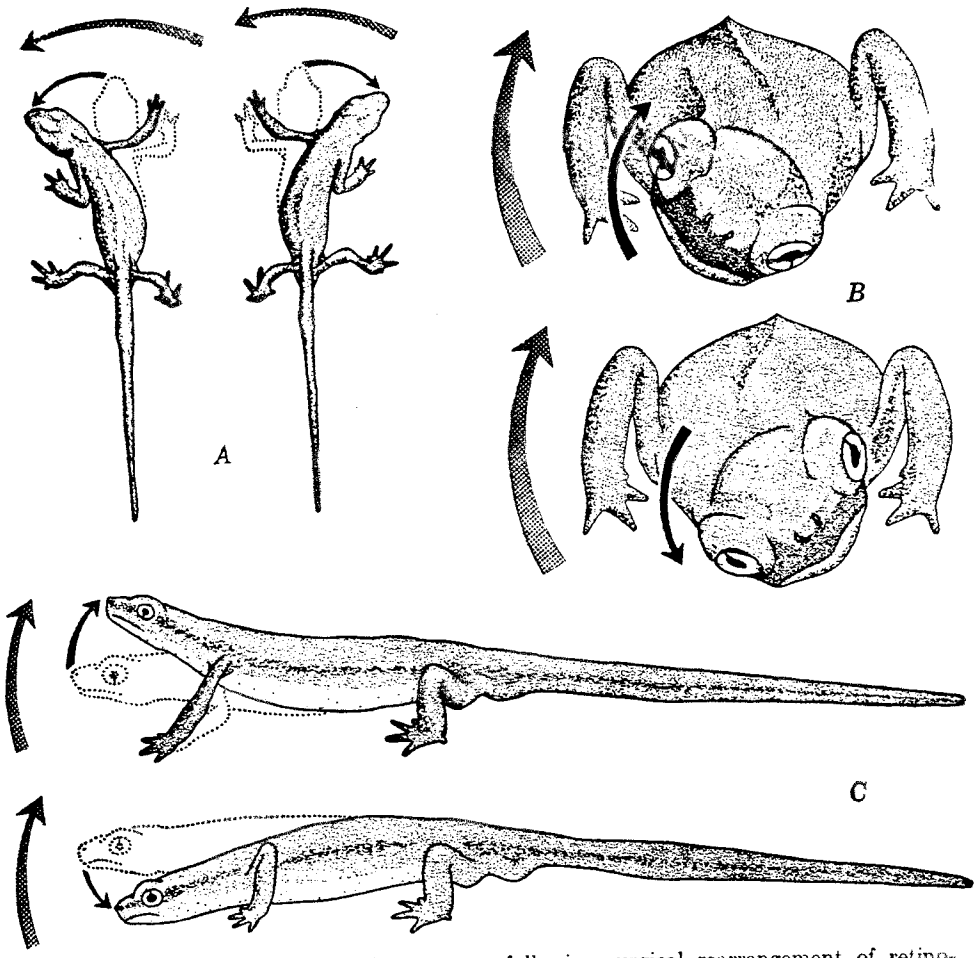

FIG. 2. Reversal of optokinetic responses following surgical rearrangement of retinocentral relations. The normal pursuit phase of the response is shown, along with the reversed pursuit reactions for the three primary planes, as caused by (1) 180-degree rotation of eyes, (2) dorsoventral inversion, (3) nasotemporal inversion, and (4) cross union of the optic nerves. Large arrows indicate the actual direction of movement of the visual field. Reversal in *A* caused by 1, 3, and 4; in *B* by 1, 2 and 4; and in *C* by 2 and 3. More complicated correlations are obtained by 90-degree rotation and by combinations of eye rotation, inversion, and optic-nerve cross union.

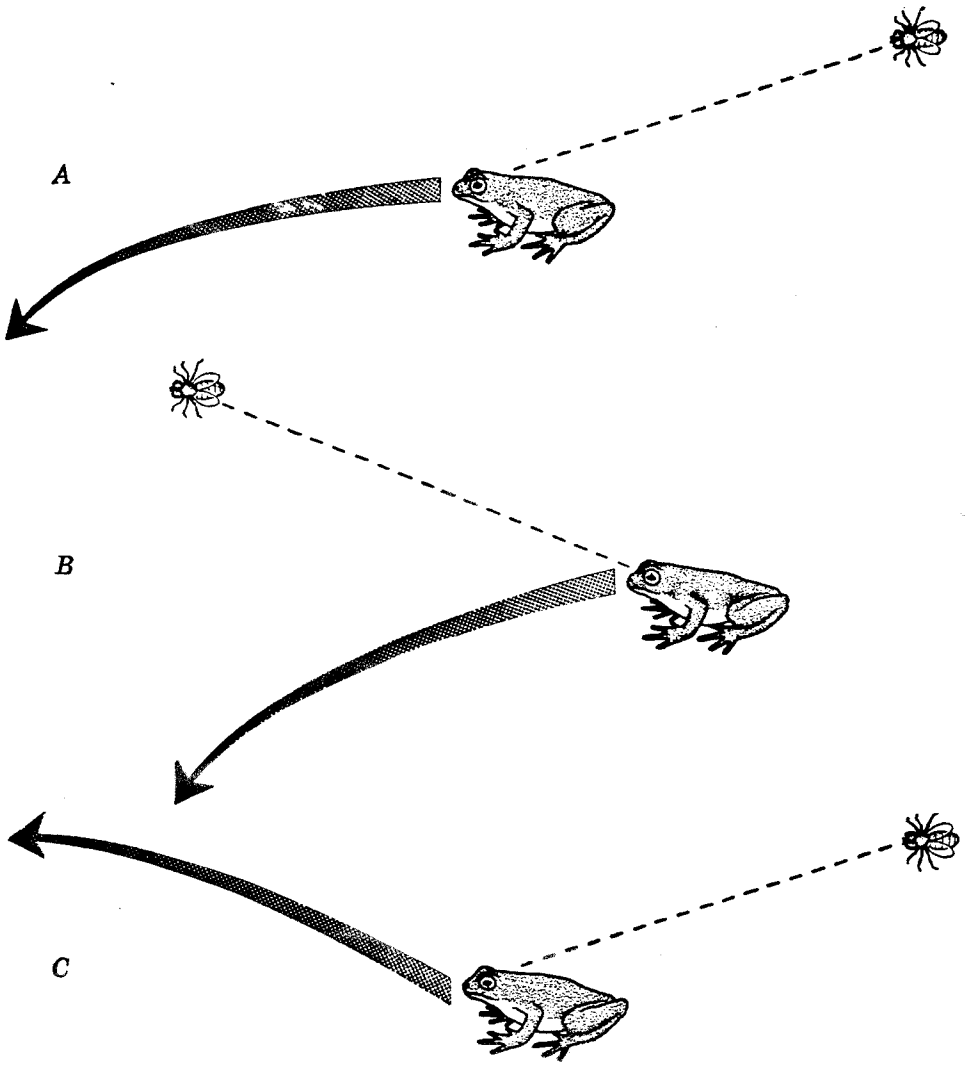

FIG. 3. Sample types of error in spatial localization of small objects following rotation and inversion of the eye. *A:* With eye rotated 180 degrees, frog strikes at a point in the visual field diametrically opposite that at which the lure is actually located. *B:* After dorsoventral inversion of the eye, frog strikes correctly with reference to the nasotemporal dimensions of the visual field, but inversely with reference to the dorsoventral dimensions. *C:* After nasotemporal inversion of the eye, frog strikes correctly with reference to the dorsoventral dimensions of the visual field, but inversely with reference to the nasotemporal dimensions.

corresponding points in the opposite sector of the visual field from that in which the bait is located (Fig. 3*A*). The animals also exhibit persistent circus movements. The maladaptive responses persist indefinitely without correction. This refractoriness to reeducation suggests, in itself, that the structure mediating these reactions is not organized by the learning process.

Surgical rotation of the eye can be combined with severance of the optic nerve (Sperry, 1942*a*, 1943*c*, 1944). This results in blindness for a period of approximately 4 weeks, during which time the fibers regenerate from the retinal stump and eventually reestablish central synaptic connections in the brain. Histological examination has shown that the optic axons, in the course of regeneration, commonly become intertangled with one another in the scar region, with a thorough disarrangement of the original fiber pattern (see Fig. 5*A* and *B*). Nevertheless the formation of synaptic relations in the centers does not follow a confused or random pattern. Instead an orderly restoration of the original functional relations between retinal fields and brain centers is achieved. This is evident in the recovery of systematically reversed visuomotor reactions directly correlated with the rotated position of the retina. Inasmuch as recovery leads to maladaptive reactions, which persist without correction, the patterning of the synaptic relations can hardly be ascribed to functional adaptation.

If the blood supply to the retina is sufficiently impaired in this operation, the retina degenerates and in the urodeles a new retina is regenerated. In the course of about 2½ months, new optic axons grow centrally from the ganglion cell layer of the regenerated retina (Fig. 5*D*). These new optic fibers form central reflex relations within the optic lobe in the same orderly way as do those of cases in which the original axons regenerate directly from the point of severance.

The amphibian eye can also be transplanted to the orbit on the opposite side of the head with only one of the primary retinal axes inverted instead of both, as diagrammed in Fig. 1*C* and *D*. In an eye transplanted to the contralateral orbit, the optic fibers of the original retina (or of the newly regenerated retina if the original retina degenerates) establish central synaptic connections in the same systematic manner as described in the previous experiments (Sperry, 1945*a*). Following recovery of vision by the transplanted eye, the animals behave as if the visual field were inverted in one dimension, corresponding to the actual inversion of the retina. Responses are properly directed with reference to the axis of the eye correctly oriented, but they are reversed with reference to the inverted axis (see Fig. 3*B* and *C*). When the eye is inverted on intermediate axes or rotated through intermediate angles, the visuomotor coordinations become misdirected accordingly. Similar results have been obtained with salamander embryos prior to the initial ingrowth of the optic axons (Stone, 1944).

The optic nerves can be cut and crossunited to the nerve stumps of the opposite eye in such a way that, after nerve regeneration, the retina becomes projected onto the opposite side of the brain from that with which it is normally connected (see Fig. 4). The consequent visual responses indicate that the part of the visual field viewed through either eye appears to the animal as if it were being seen through the contralateral eye (Sperry, 1945*a*). There is reversal of optokinetic reactions around the dorsoventral and rostrocaudal axes of the body, forced circus movements on these same axes, and errors of spatial localization with reference to the left-right aspect of the visual field. These visuomotor responses remain uncorrected by experience.

Following each of these surgical rearrangements, the neuronal connections are laid down in the same prefixed manner. The optic axons may be growing into the brain for the first time in embryonic development, they may arise from newly proliferated neuroblasts in a regenerated retina in the adult,

or they may be old fibers regenerating directly after transection. Regardless of whether the retina is upright or upside down or whether the optic tracts are connected to the proper optic lobe or to the opposite lobe, the central reflex associations formed by the ingrowing optic fibers are established in the same systematic pattern. These functional relations are formed in a predetermined fashion without regard for the adaptiveness of the functional effect. Extension of the experiments to teleost fishes (Sperry, 1948*b*, 1949) has yielded similar results.

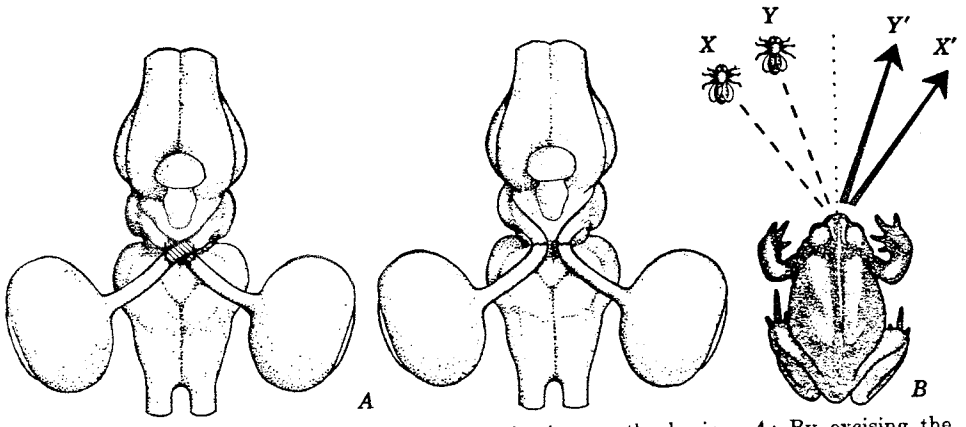

FIG. 4. Contralateral transfer of retinal projection on the brain. *A:* By excising the optic chiasma and cross-uniting the four optic nerve stumps as diagrammed, the central projection of the two retinas can be interchanged. *B:* After optic-nerve regeneration the animals respond as if everything viewed through either eye were being seen through the opposite eye. For example, when a lure is presented at X or at Y, the animal strikes at X' or at Y', respectively (see also Fig. 2).

A lesion made in a given quadrant of the optic lobe of the midbrain of a normal frog produces a "blind" area in the corresponding quadrant of the visual field (Sperry, 1944). The animal then makes no response when the lure is presented in the "blind" area. This fact, bolstered by the anatomical studies of Stroer, indicates that the retina normally has an orderly projection upon the optic lobe. If, in addition, the eye has been surgically rotated, the "blind" area shifts correspondingly. The regenerated optic fibers, despite their mix-up in the scar region, apparently manage to restore functional connections with the same areas of the optic lobe with which they were originally connected.

These experiments all indicate that fibers arising from a given locus of the retina are predestined to form their central synapses with neurons located in a specific locus. Each retinal point has its corresponding point in the optic lobe. The formation of associations is governed strictly by anatomical relations, not by the adequacy of the functional effects.

Although the mechanisms involved in the establishment of these central reflex relations remain somewhat obscure, several inferences may be drawn from the evidence now available. The general direction of growth of the optic tract as a whole might be due largely to mechanical guidance. On the other hand, it is clear that the arrangement of terminations within the optic lobe itself could hardly be explained in this way. The frayed and disoriented condition of the nerve stumps, the erratic and tortuously intertwined course of the optic axons through the nerve scar, the absence of preconstructed pathways both in the embryo and in animals with newly

regenerated retinas, all indicate that mechanical guidance could not possibly be responsible for the patterning of synaptic relations. Nor does the assumption of a precise timing of axon outgrowth help the situation. All the fibers start their outgrowth at approximately the same time from the cut surface of the nerve stump, and the required mechanical alignment in the substrate of the optic pathway is lacking.

It is obviously necessary that optic fibers arising from different retinal loci be distinguished from one another in the centers. Were the optic axons all alike in character, there would be no basis — with mechanical guidance, timing, and functional adaptation

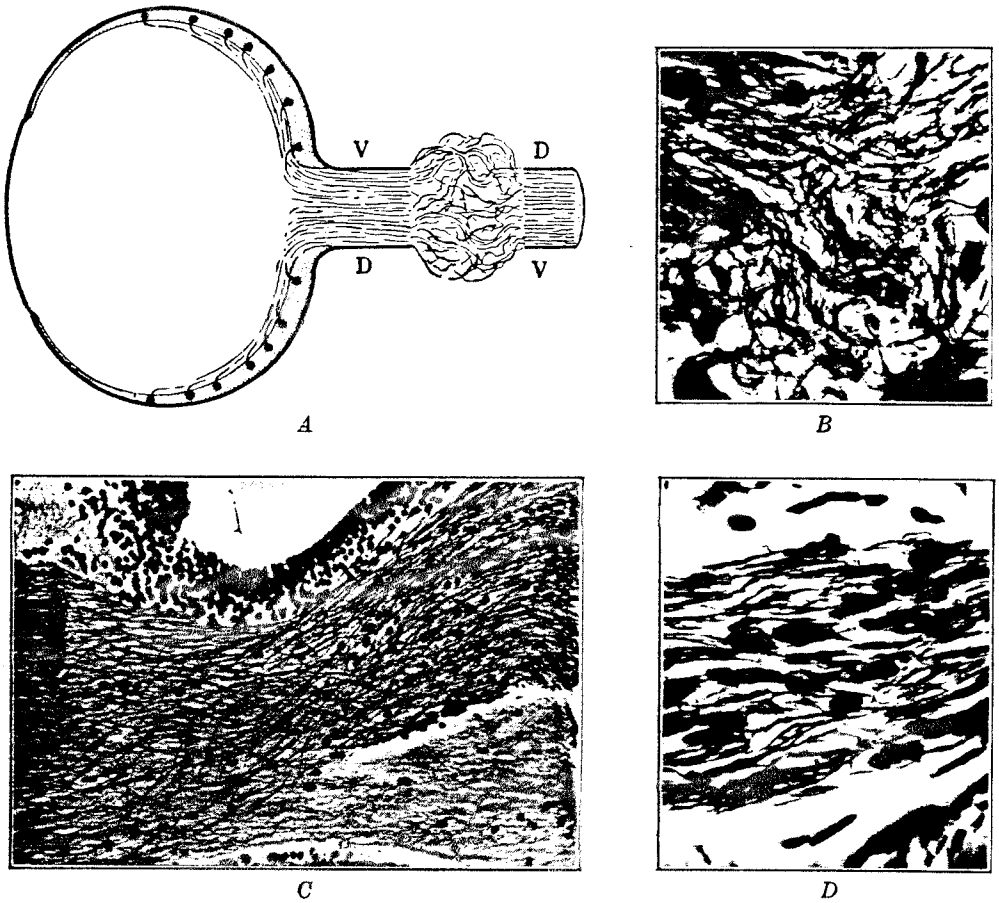

FIG. 5. Optic nerve regeneration (from Sperry, 1943*c*, 1945*a*). Despite extreme intermixing of the regenerating fibers in the scar region and despite rotation or inversion of the opposing nerve stumps as diagrammed in *A*, the fibers reestablish their functional associations in the brain in an orderly manner. The photomicrographs show the interwoven, nonparallel course of the regenerated optic fibers in animals that had shown orderly functional recovery. *B:* Optic-nerve regeneration scar in a newt. *C:* Regenerated optic chiasma in a frog, following contralateral transplantation of the eye. *D:* Regenerated optic nerve of a newt, following retinal degeneration and regeneration.

all ruled out — for a selective arrangement of synaptic relations. Consequently there must be some kind of qualitative specificity among the ingrowing axons determined by the loci of the retinal field from which they arise. Not only the optic axons but also the secondary neurons of the optic lobe with which they connect must each have a distinctive character. If the secondary central neurons were all alike, there would again be no way of attaining order.

The qualitative specificity must parallel the topography of the retinal field. This means a true "field" distribution of qualitative properties among the retinal ganglion cells from which the optic fibers originate and among the tectal neurons on which they terminate. It means, furthermore, that the retina must be differentiated with respect to at least two axes of the eyeball in order that each locus may have biochemical properties different from those of all other loci.

How and when differentiation is established in the retina and optic lobe are questions proper to embryology and cytochemistry. Of more direct interest to us is how this neuronal differentiation affects the patterning of synaptic associations. The simplest assumption is that this patterning is governed by biochemical affinities and incompatibilities between the central neurons and the ingrowing optic axons.

As the optic fibers invade the optic lobes, they have opportunity to make many contacts among the dense population of nerve cell bodies, dendrites, axons, blood capillaries, glial cells, and other optic fibers that have preceded them into the area. Of these many contacts only a few result in the formation of synaptic endings. The fiber tips grow around and past glial cells, capillaries, axons, and the majority of dendrites and nerve cell bodies they happen to encounter. But, when they reach the appropriate part of the optic lobe, they meet neurons whose physicochemical nature is right for the formation of synaptic end feet. It is likely that a single optic axon terminates on a large number of secondary neurons whose dendrites may spread over a considerable portion of the optic tectum but whose cell bodies occupy only a small area. The cells in the center of such an area would receive the greatest number of terminals from that particular axon. The terminations of neighboring cells would presumably overlap considerably, but the projection of each retinal locus would still be centered about a unique focal point in the field of tectal neurons. The relative degree of overlap has apparently decreased along with a decrease in the spread of the axonal and dendritic arborizations during evolutionary development.

Similar developmental phenomena could account for the orderly projection of the retinal field upon the striate cortex in mammals, although further complications arise from the overlap of the visual fields and the partial crossing of fibers at the chiasma. The close termination of optic fibers from congruent points of the two retinas is a difficult feature to explain. In this regard it is noteworthy that the geniculate cells on which the fibers from the two eyes terminate, tend to migrate into separate layers.

Central Synapses of the Vestibular Nerve

From the nature of vestibular reflexes it is evident that the fibers that supply the crista of any one semicircular canal must have different reflex relations in the hindbrain from the fibers supplying the crista of either of the other canals. Similarly the fibers to each of the approximately seven separate end organs of the amphibian labyrinth must have their own special linkages. There exists a certain overlap, undoubtedly, in the central relations of the fibers of some of these separate end organs. At the same time, however, fibers supplying different parts of a single end organ, such as those to the utricular macula, must have differential central associations to match their functional diversity.

Complete bilateral severance of the VIII cranial nerve root along with the root fibers

of VII was performed in the adult frog, *Hyla squirella* (Sperry, 1945*b*). The root fibers were able to regenerate centripetally into their nuclei in the medulla, as indicated by recovery of function and by histological examination (see Fig. 6). The profound disturbance of equilibration caused by section of the vestibular nerves was repaired in the experimental cases but not in control cases in which regeneration was prevented. Regeneration restored the various reflex responses to angular acceleration and tilting of the animals in the three primary planes of the body (Fig. 7). Evidently the different neuron types among the heterogeneous collection of divided sensory root fibers were

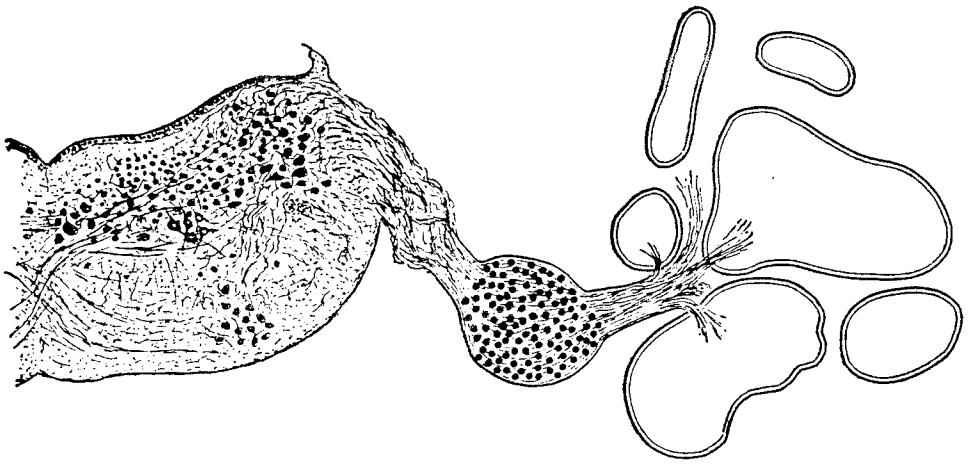

FIG. 6. Diagrammatic section through regenerated vestibular nerve root at its entrance into the medulla. Fibers connected to the various sensory endings in the semicircular canals, the utriculus, the sacculus, and the lagena become intertangled at the point of nerve section, but they nevertheless reestablish central reflex relations in a systematic pattern.

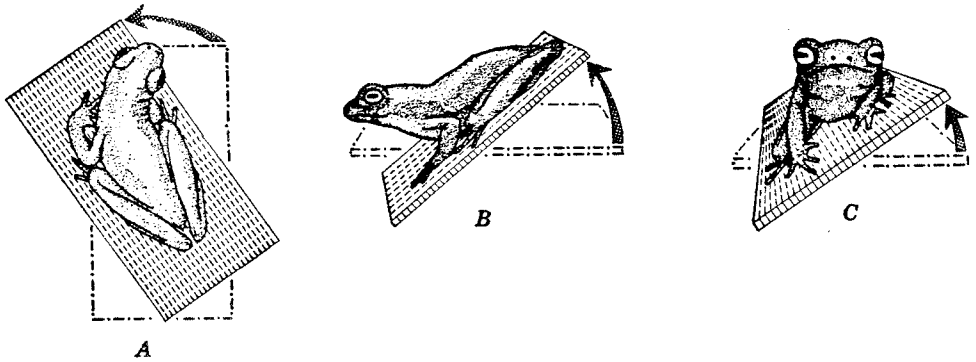

FIG. 7. Vestibular reflexes. Different patterns of labyrinthine stimulation produced by rotating and tilting animals in the three primary planes of the body elicit specific reflex responses and postures which, along with other vestibular responses, furnish good criteria of the orderliness of functional recovery following regeneration of the vestibular nerve root.

able to reestablish functional relations with the secondary central cells in a discriminative manner. Judging from the number of different kinds of sensory end organs supplied by nerves VII and VIII, one would estimate that at least eleven distinct classes of fibers regenerated from the point of transection with ample opportunity for a chaotic interspersion into abnormal pathways, and with very little chance for particular fiber types to be directed to their proper central terminations by mechanical factors alone. Nevertheless reflex relations were restored in an orderly fashion.

There was no indication of learning during the period of recovery. Nor were the recovered reactions impaired by decerebration. Regeneration of the VIII root in tadpoles was also found to restore the compensatory vestibulo-ocular reflexes, even though the optic nerves in these animals had been previously excised so that the formation of proper reflex relations was functionally useless. The normal development of the vestibulo-ocular reflexes in blinded animals, including amphibians (Sperry, 1946), pigeons (Mowrer, 1936), chickens (Kuo, 1932), and rabbits (Nasiell, 1924), is further evidence that learning is not important in patterning the synaptic associations of the vestibular nerve.

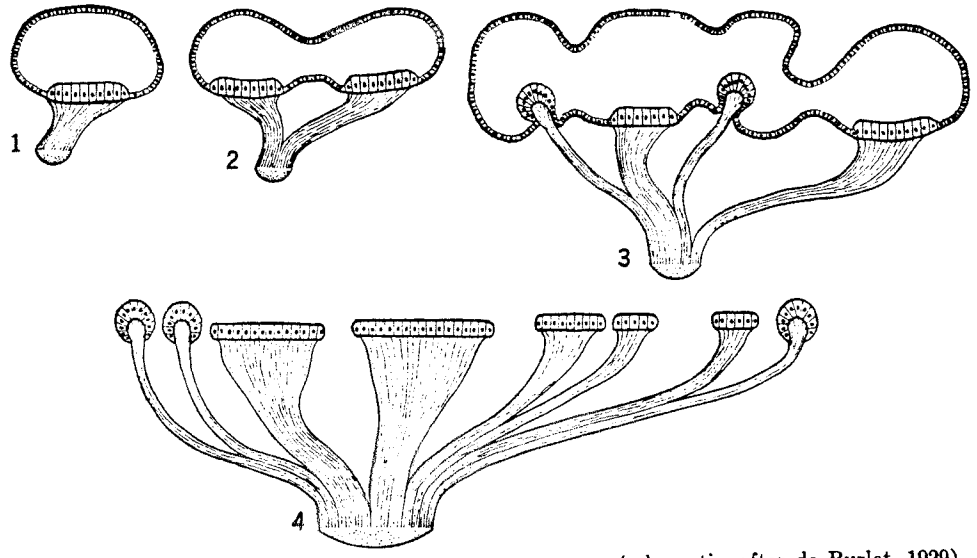

FIG. 8. Differentiation of the vestibular sense organs (schematic, after de Burlet, 1929). As the simple otic vesicle enlarges and transforms into the complicated adult labyrinth, the single patch of sensory epithelium, with nerve fibers attached, becomes subdivided into many parts which undergo independent differentiation to form the various adult sense organs.

The strict correlation between the behavior of the vestibular fibers and their peripheral connections suggests a causal relation. From descriptions of the development of the vestibular apparatus and its nerve supply (Harrison, 1936; de Burlet, 1929), it appears that here, as in the visual system, the end organs probably lead the way in the matter of differentiation and induce specificity in their associated nerve fibers. Early in development the vestibular fibers are all connected to a single patch of sensory epithelium. From this single patch of cells are pinched off approximately seven separate parts that become the various end organs of

the labyrinth (see Fig. 8). Presumably, as the parts undergo differentiation, they induce a parallel differentiation in the neurons connected to them.

Unlike the situation in the retina, the sensory cell bodies and dendrites are not embedded within the end-organ tissue. It must be supposed, therefore, that specification is imposed on the neurons through their terminal connections. Specification of this kind is well authenticated in the case of the nerves to the limb muscles (Weiss, 1941*d*). The chemical or physical basis of the "induction" effects of one tissue upon the differentiation of neighboring tissue has long been a matter of conjecture. It would appear to involve some type of contact or chain-reaction process. For further discussion of the general problem, see Weiss (1947).

In order that the vestibular fibers may form proper synaptic relations among the central neurons, it is necessary that the secondary neurons also be heterogeneous in character. This could be achieved through self-differentiation or possibly, in part, through induction effects from axon connections with the motor system. It is a general rule of early development (Coghill, 1929, 1930*b*; Herrick, 1939; Windle, 1944, etc.) that the association neurons join with the motor neurons before synapses are established with the sensory fibers. Hence it is possible that axon connections with the already differentiated cells of the motor system influence the refined stages of specification among the association cells.

Genesis of Cutaneous Local Sign

Most vertebrates including man are able without visual aid to localize a tactile stimulus applied to the skin anywhere on the body. Even the frog localizes with considerable accuracy. Mild cutaneous stimuli applied at different points about the back, thorax, hip, thigh, knee, shank, and foot will evoke responses well aimed at the points stimulated. The false localization that follows misregeneration of nerves into foreign regions of the skin shows that the local sign quality depends upon the central relations of the cutaneous fibers. For normal localization the central connections of each fiber must suit the particular peripheral area that the fiber innervates. In a sense the map of the body surface must be reflected in the central circuits.

It has been widely assumed that cutaneous local sign is acquired by experience. Holt (1931) presented a scheme, based on the theory of neurobiotaxis, by which this "education of the sensory surfaces" might conceivably take place (see pp. 237 and 263). A later study on the cross union of sensory nerves in the rat (Sperry, 1943*a*), however, has furnished strong evidence of an inherent organization in these animals. Inherent organization is also the rule in the development of cutaneous local sign in amphibians.

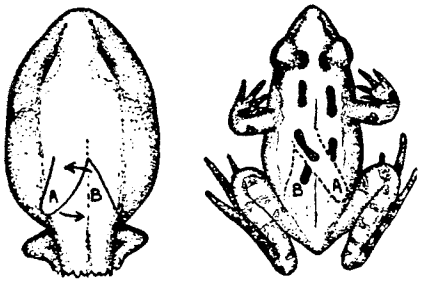

FIG. 9. Contralateral translocation of skin flaps. Skin translocation across the midline of the back in frog tadpoles results in contralateral misdirection of localizing reactions after metamorphosis.

In the frog the hind-limb localizing reactions survive cord transection, indicating that they are organized mainly at the spinal level. Skin flaps with their original innervation largely intact can be transplanted across the midline of the back (Fig. 9) of the metamorphosing tadpole, prior to any experience with localizing responses. After metamorphosis is complete, the frogs display localizing reactions that are misdirected to the contralateral side. Stimuli applied to the translocated skin elicit wiping reactions on the

opposite side falsely aimed at the original site of the skin flap.

It is also possible by surgery to cross-connect the dorsal roots of the hind-limb nerves to the opposite side of the cord (Fig. 10). When the operations are performed in tadpole stages, the root fibers regenerate to form reflex relations with the motor centers of the contralateral limb. After metamorphosis cutaneous stimuli applied to the foot result in characteristic reflex reactions of the opposite leg, while the foot stimulated remains motionless (Sperry, 1947*b*). Cutaneous local sign in the spinal hind-limb area can hardly be ascribed, therefore, to functional adjustment.

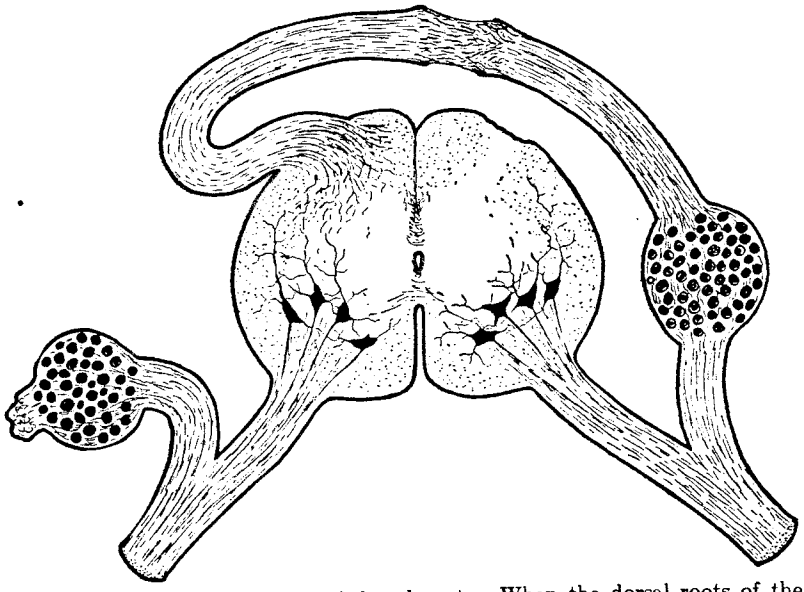

FIG. 10. Contralateral cross union of dorsal roots. When the dorsal roots of the hind-limb nerves are crossed in the manner indicated, the regenerating sensory root fibers establish functional relations with the spinal centers of the contralateral limb similar to those that they establish with the ipsilateral limb centers. This selective formation of the central reflex connections cannot be attributed either to mechanical guidance or to functional adjustment.

Stimulation of different points about the head and face within the area innervated by the large trigeminal nerve will elicit specific wiping reactions of the ipsilateral forelimb in frogs and toads and specific withdrawal responses in the urodele amphibians. In frog tadpoles the ophthalmic branch of the trigeminal nerve on one side can be crossed peripherally to the contralateral ophthalmic nerve (Fig. 11). After metamorphosis the wiping responses are misdirected to the wrong side of the head; e.g., stimulation of the left ophthalmic area elicits wiping reactions of the opposite forelimb aimed at the right ophthalmic area (Sperry and Miner, 1949). When the ophthalmic nerve is crossed into the contralateral mandibular nerve, stimulation of the ophthalmic-innervated mandibular region evokes reactions of the opposite forelimb misdirected not only to the opposite side but toward the ophthalmic instead of the mandibular area (Miner, 1949). The ophthalmic branch of V has also been crossed in efts to the ipsilateral mandibular branch of V, the central root of which was then cut and allowed to regenerate into the

brain (Fig. 12). The regenerating root fibers reestablished orderly relations, but, because of the peripheral nerve cross, the resultant responses were functionally maladaptive. Pricking the underside of the jaw caused the head to be depressed more strongly against the point of the needle (Sperry and Miner, 1949). Thus, in regeneration as well as in development, the central circuits became patterned in an orderly manner with reference to certain anatomical relations but without regard for functional adaptiveness.

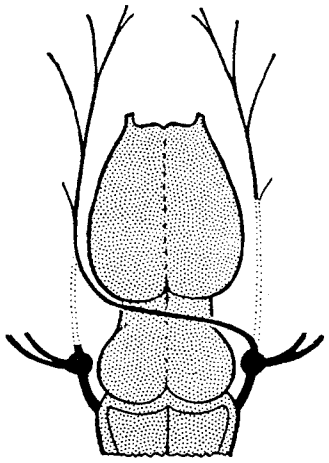

FIG. 11. Contralateral cross union of ophthalmic nerves (from Sperry and Miner, 1949). The left side of head and snout become reinnervated by the crossed right ophthalmic nerve in larval stages. After metamorphosis localizing responses are misdirected to the wrong side of the head.

Good functional recovery of the transected root V following haphazard intertangling of fibers in the scar (Fig. 13) shows that the central patterning is not merely the product of an orderly spacing and timing of fiber ingrowth. Even when the cut root of V is made to regenerate into the brain over the central pathways of nerve VII (Fig. 14) the local sign properties of the trigeminal cutaneous field are correctly restored. It is apparent that in the latter case there is no opportunity for the individual fibers to follow their old channels to the original central terminals. The dorsal roots of the hindlimb nerves are exceptional in this regard, in that full recovery of functional specificity is not obtained, save in a small percentage of cases. After recovery the foot reactions are less differentiated than normal, with the more refined wiping responses usually lacking. Possibly in this instance the timing of fiber ingrowth may be involved in the adjustment of central linkages.

The conclusion is evident that the sensory neurons supplying different cutaneous areas must differ in character. Fibers from different loci must somehow be distinguishable in the centers. A correlated specificity among the second-order neurons is also indicated.

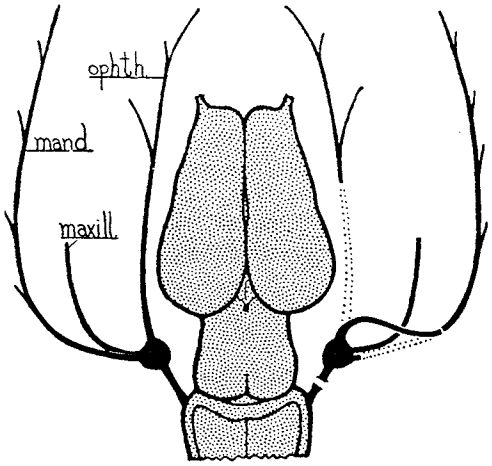

FIG. 12. Cross union of ophthalmic and mandibular nerves with section of root V (from Sperry and Miner, 1949). After recovery, the eft's withdrawal responses to stimulation of the mandible are reversed.

The central neurons must be distinguished in accordance with their various efferent relations. The orderly patterning of central reflex relations then becomes explicable, as in the visual and vestibular systems, on the basis of differential chemoaffinities between the sensory and central neurons.

Furthermore the specificity of the sensory fibers must correspond to the topography of

the cutaneous field, reflecting the spatial interrelations of all cutaneous points. Finally the experiments indicate a highly refined, fieldlike differentiation of the entire integument, itself. Without this it would not be possible to obtain the neat adjustment of central-peripheral linkages.

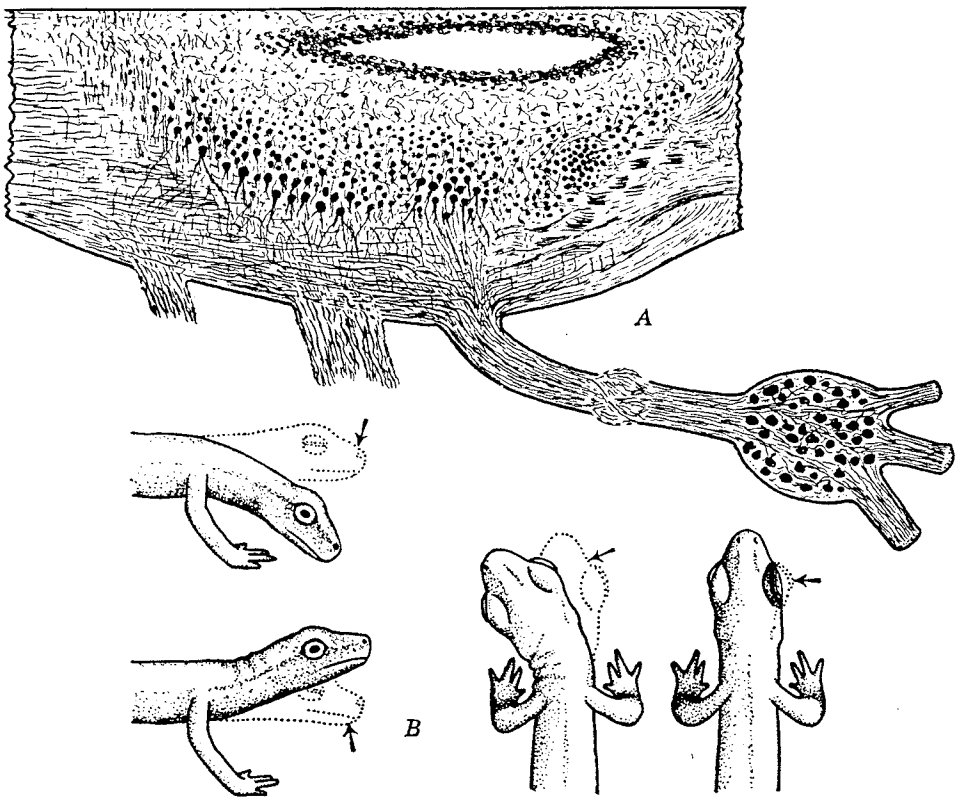

FIG. 13. Centripetal regeneration of trigeminal nerve root. *A:* Diagrammatic frontal section through the regenerated Vth nerve root at its entrance into the medulla. *B:* Different kinds of avoidance or withdrawal reflexes mediated by regenerated fibers of V are consistently restored in an orderly manner.

Conceivably the parallel specification of the sensory neurons and the integument might be achieved by independent self-differentiation. In this case the attainment of proper peripheral connections would call for a selective outgrowth of the sensory fibers, each to its correct cutaneous locus. The required linkages could also be established through a relatively indifferent outgrowth and termination of the sensory fibers, followed later by induction of specificity in the fibers as a consequence of their connections with the cutaneous field.

The evidence at present tends to favor the latter possibility. Fibers of the thoracic nerves can be made to connect atypically with limb instead of thoracic skin by extirpating the sensory ganglia of the limb, or by transplanting limbs into the midthoracic region of the back. The sensory fibers then develop central linkages suited to the particular part of the limb integument in which they happen to terminate (Miner, 1949). A similar specification of sensory fibers mediating the corneal reflex has been inferred from

the results of transplanting extra eyes to atypical locations about the head (Weiss, 1942; Kollros, 1943). Evidence of this specification failed to appear in the ophthalmic to mandibular nerve crosses cited above, but the fiber specificity may already have become irreversibly determined. Even in mid-larval stages in the frog tadpole the ophthalmic and mandibular divisions of nerve V have been found to retain their original central associations after connecting with foreign integument (Miner, 1949).

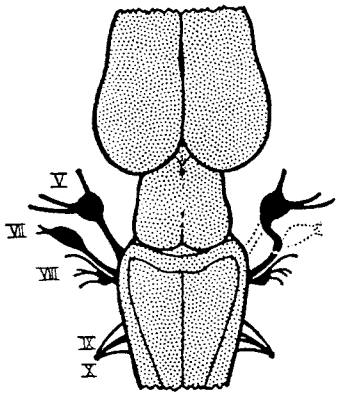

FIG. 14. Cross union of V and VII (from Sperry and Miner, 1949). The trigeminal fibers regenerate into the brain via pathways of VII.

The evidence thus far does not preclude the probability that the sensory ganglia of the various cranial and spinal nerves undergo some degree of differentiation independently of contact with the integument. But the refined specification necessary for localizing different points within a given dermal segment is apparently dependent upon inductive effects imposed upon the nerve fibers by the differentiating skin. An extensive overlap in the terminations of neighboring cutaneous fibers both in the periphery and in the centers must be assumed.

An additional dimension of differentiation among the cutaneous fibers is required for the different modalities of pain, touch, and temperature. Presumably the "local sign" specificity is superimposed upon this more general modal differentiation. How the modal differentiation is controlled and how the different classes of fibers become distributed in proper proportions to the cutaneous field are problems thus far untouched.

Central Reflex Connections of the Proprioceptive Fibers

The sensory neurons supplying the proprioceptors of the musculature develop specific reflex relations in the centers suited to the particular muscles that the neurons innervate. This is necessary to the regulation of posture and of motor coordination. Functional specificity is manifest in stretch reflexes like the knee jerk or ankle jerk, in which sudden stretching of the muscle calls forth a reflex contraction selectively centered in the same muscle (Lloyd, 1946).

It has been shown (Verzár and Weiss, 1930; Weiss, 1937*a*) that the reflex properties acquired by the proprioceptive fibers of different limb muscles depend upon specification of the fibers by their muscles. When divided fibers of a limb nerve regenerate into the muscles of an extra limb transplanted in the vicinity of the normal limb, their reflex relations become adjusted to suit the particular muscles with which they happen to connect. After recovery passive stretching of different muscles in the extra limb elicits a contraction of the stretched muscles, and also of the homologous muscles of the normal limb. The functional relations thus automatically become adjusted to the pattern of the peripheral connections.

This cannot be ascribed to learning, because the movements of the extra limb serve no purpose. In some cases they are positively detrimental to the animals. The assumption that fibers connect selectively with particular muscles has also been ruled out. The only remaining possibility is that the character of the proprioceptive neurons is determined by inductive effects from the muscles.

In regeneration of the dorsal roots of the hind limb, proprioceptive fibers reestablish

connections along with the exteroceptive cutaneous fibers. The proprioceptive reflex relations are restored in a selective manner. If the dorsal roots of the right leg are crossed into the spinal centers of the left leg, the reactions of the left leg in response to stimuli applied to the right leg are conditioned by the posture of the right leg. For example, as illustrated in Fig. 15, a tactile stimulus to the toes of the right foot may evoke either an extensor kick or, conversely, a flexor withdrawal of the opposite left foot, depending on whether the right leg happens to be flexed or extended. Similar influence of the leg's posture upon its own responses is observed after ipsilateral regeneration of the divided dorsal roots.

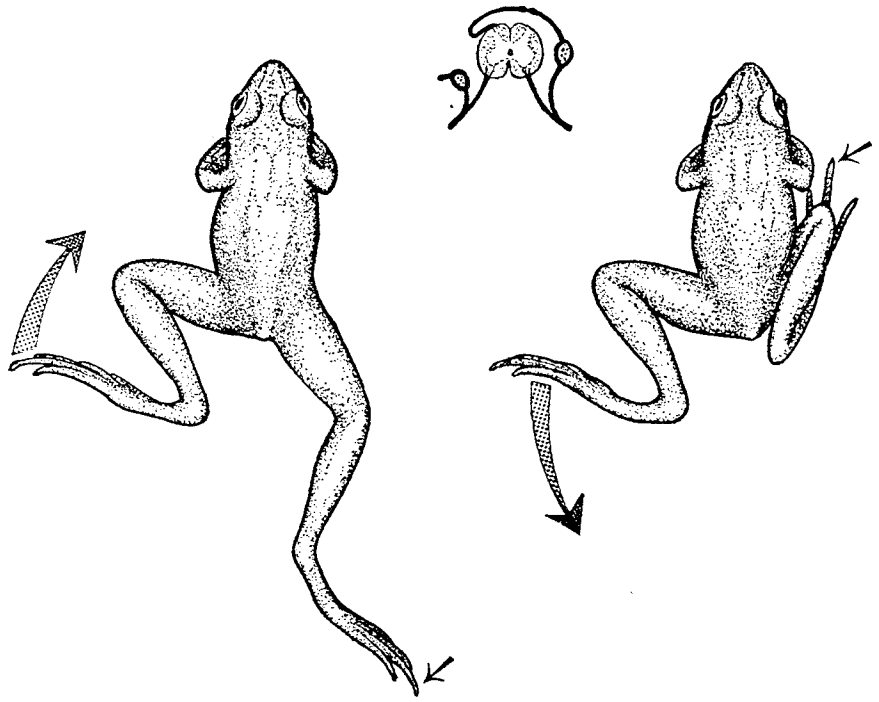

FIG. 15. Systematic reestablishment of proprioception. After crossed regeneration of the dorsal roots, the posture of the right limb comes to condition the type of reflex responses made by the contralateral limb in the way it usually conditions its own responses. Stimulation of the right foot (small arrows) causes flexion or extension of the left leg, respectively, depending on whether the right leg is extended or flexed.

The central patterning of the proprioceptive reflexes following centripetal regeneration of the root fibers can be readily interpreted in terms of chemoaffinity. We need only assume that the ingrowing fibers from different muscles form synapses with different classes of central neurons in a discriminative manner.

The interpretation is not so easy, however, in the earlier limb-transplant experiments of Weiss in which the original central connections were left intact. On the assumption that the central connections remained fixed, the orderly establishment of the myotatic responses after disarrangement of the peripheral connections was taken to mean that specific connections are not basic to central

nervous integration. It was inferred that the central-peripheral selectivity must be based instead upon physiological "resonance" phenomena (see p. 273) and that the muscular specification of the proprioceptive fibers modulates the quality of the impulses the fibers transmit. In order to bring these results into harmony with our present connectionist interpretation it is necessary to postulate that the existing central synapses undergo some kind of trophic breakdown and rearrangement in response to the new peripheral relations.

The foregoing evidence on the development of sensori-central connections reveals a recurring pattern throughout. In the visual, vestibular, cutaneous, and muscular proprioceptive systems, the end-organ tissue apparently undergoes the primary differentiation and then induces local specificity in whatever sensory fibers are in contact with it. The induced fiber specificity then determines in turn the pattern of synaptic associations to be formed in the centers. It seems likely that this same type of "peripheral regulation of central synapsis" may occur also with other tissues such as the cochlea, the tendons, the joints, the ligaments, the facias, the periosteum, etc., the sensory innervation of which possesses local functional specificity. On the foregoing scheme neuronal connections neatly designed for adaptive function can be laid down between the sense organs and the centers by purely developmental forces.

Motor Neuron Associations

The central relations of the primary motor cells must be adjusted to suit their peripheral connections in the musculature. The experiments of Weiss (1922–1937) dealing with the factors controlling selectivity between musculature and nerve centers in amphibians antedate most of the investigations already cited. Much of the more recent work has been stimulated directly by these pioneer investigations and the challenging conclusions to which they led. Their consideration has been delayed to this point because the results on the motor system involve relations and interpretations somewhat more complicated than those dealing with the sensory systems.

It is conceivable that the motor neurons might attain their proper peripheral connections by means of selective axon outgrowth. A given neuron might seek its appropriate muscle. This would require a previous specification of the motor cells into as many different types as there are muscles to be innervated. Actually, however, the outgrowth and termination of the spinal motor axons in amphibians has been reported (Piatt, 1940; Weiss, 1937*b*) to be entirely nonselective, both in regeneration and in development. Under normal conditions some selectivity of axon termination would seem to be assured by the proximodistal order in which the limb segments develop (Saunders, 1947) and by the chronological order in which the neurons differentiate and send forth their axons. Nevertheless considerable freedom remains within any limb segment, and, since proper muscular coordination is achieved even with highly random outgrowth and termination in the periphery, it follows that the central relations must be adjusted secondarily to suit the peripheral innervation. Even when divided motor axons are forced to regenerate into entirely foreign muscles, the timing of the central discharge becomes adapted to the new terminals (Weiss, 1928, 1936, 1941*d*).

This adaptation in the timing of the central discharge is not achieved by the learning process. If limbs are transplanted to dorsal positions such that their movements are of no value to the animal, the central-peripheral adjustments occur in the usual systematic manner. This is true even when the limbs are transplanted to the contralateral side and reversed in such a way that the movement of the limbs tends to push the animal backward when it attempts to go forward and vice versa, as indicated in Fig. 16 (Weiss, 1941*d*). Similar effects are ob-

tained when the limb transplantations are made in prefunctional stages (Brandt, 1925, 1940; Detwiler, 1925; Weiss, 1941*d*), showing that these relations are patterned initially through developmental forces and not through any kind of functional adjustment. Furthermore it has been shown that these motor patterns develop in the same systematic way in the absence of sensory innervation (Weiss, 1937*c*) and that they persist after decerebration and cord transection down to levels of the cord just rostral to the limb segments (Weiss, 1936). The possibility that learning or any type of functional adaptation might be responsible has thus been excluded.

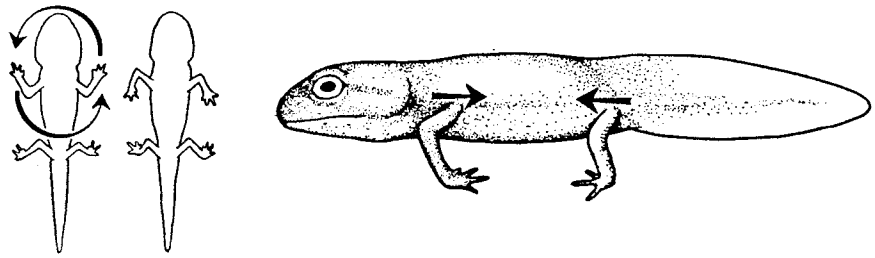

FIG. 16. Reversed locomotion after interchange of right and left forelimbs (after Weiss, 1941*d*). After prefunctional reversal of the anteroposterior axes of the forelimbs, the central coordination patterns develop in the usual way, with the result that the transplanted forelimbs oppose the normal action of the hind limbs.

The motor neurons must somehow be distinguished from one another in the centers according to the muscles they innervate, because the timing of their central discharge is always adjusted on this basis. Weiss concluded that the motor neurons possess some constitutional (presumably biochemical) specificity induced in them by their muscles. This apparently was the first suggestion that nerves may be specified or "modulated" by their end organs. The corollary follows that each muscle of the limb must have biochemical properties all its own. Indeed it would be difficult to account for the morphogenesis of the limb musculature itself in the absence of such a differentiation.

In these experiments of Weiss it was only the end-organ connections that were disturbed. The recovery of typical muscle function with atypical end-organ connections might be accounted for by assuming either (1) that central-peripheral selectivity is independent of specific connections and is achieved instead on a physiological "resonance principle" (Weiss, 1928, 1936, 1941), or (2) that the rearrangement of muscular connections produces a compensating readjustment among the central connections (Sperry, 1941, 1943*c*). Decisive proof regarding these two alternatives is lacking. However, the latter fits in with our general chemoaffinity theory of synaptic patterning and is also favored at present because it does not necessitate any significant revision of our current views of central nervous integration. Accordingly the biochemical specification of the motor neurons may be tentatively considered to influence the type of synaptic linkages the cells will tolerate. It is presumed that the sensory and association neurons form their synapses among the motor cells originally in a selective manner, depending upon the biochemical specificity of the motor neurons. When axons regenerate into foreign muscles, the change in biochemical properties induced by the new muscles is thought to cause a trophic degeneration of the synaptic endfeet upon the motor cells. New synapses are then established according to the revised pattern of chemical affinities (see Fig. 17).

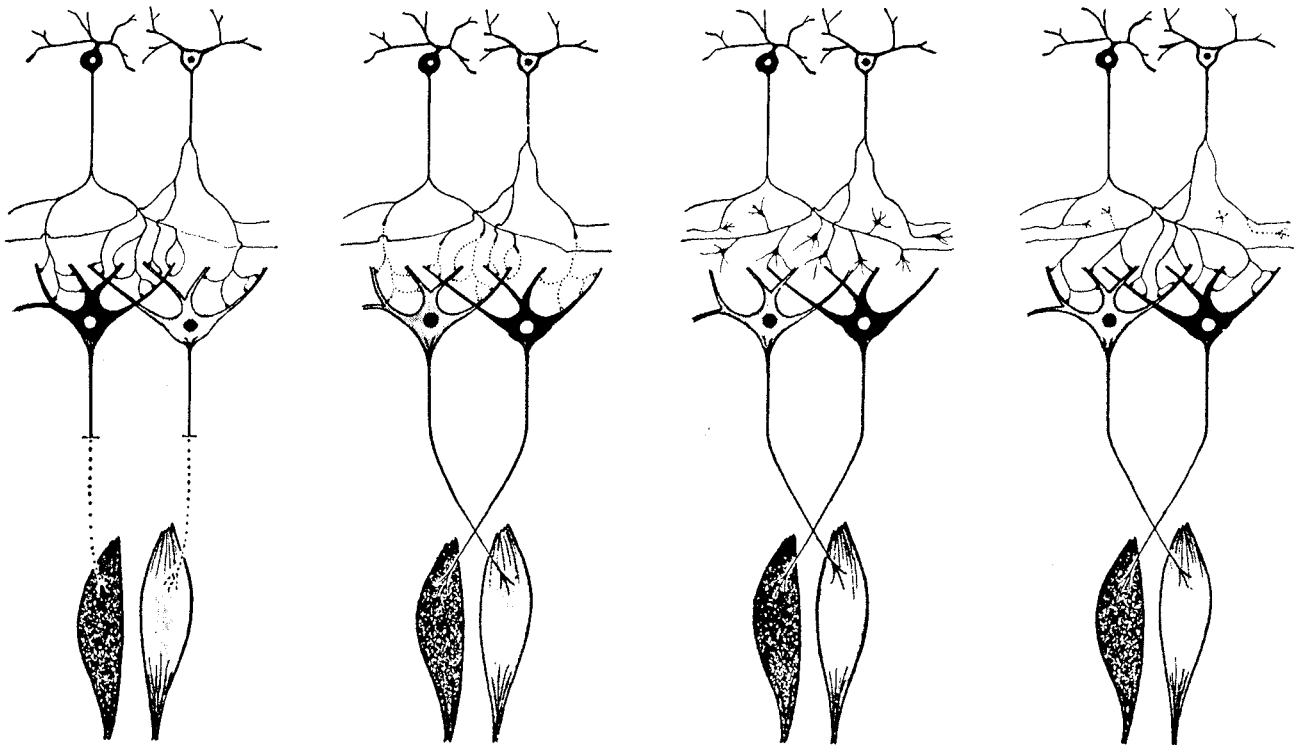

FIG. 17. Schematic representation of a possible connectionist explanation of the orderly recovery of muscle coordination that follows peripheral cross-up of nerve-muscle connections in amphibian larvae. The central synaptic associations are assumed to be established originally on a selective chemoaffinity basis. The specificity of the primary motor neurons is imposed by the musculature, and it changes when the nerves regenerate into foreign muscles, as shown by Weiss. This shift of chemical specificity is presumed to cause a breakdown of the original synaptic endings on the motor cells, followed by reestablishment of a new pattern of synaptic terminals determined by the new interneuronal affinities.

Motor reorganization of this kind occurs readily in the limbs of amphibian larvae. It goes more slowly during and shortly after metamorphosis (Weiss, 1936) and evidently does not occur at all in full-grown anurans. In the phylogenetically more ancient oculomotor system of amphibians the capacity for reorganization seems to be lost at a very early larval stage in both anurans and urodeles. Regeneration of the oculomotor nerve yields undifferentiated mass contraction similar to that obtained in mammals (Sperry, 1947*a*). Also cross innervation of the inferior oblique muscle by the nerve of the superior oblique muscle yields a reversal of wheel movements of the eye. The function of congenital supernumerary fingers in man (Weiss and Ruch, 1936) suggests that, if nerve rearrangements could be performed sufficiently early in mammals, results similar to those obtained in amphibian limbs might be demonstrated. In the rat the relations of the limb motor neurons has been found to be already irreversible at, or shortly after, birth (Sperry, 1941*j*). The newborn opossum has yet to be investigated in this respect. Where adjustment fails to occur, it may be attributed to the fact that either the motor neurons in postembryonic and postlarval stages are no longer subject to respecification, or else that such respecification no longer causes a breakdown in the existing synapses.

Apparently the cranial motor nuclei III, IV, and VI undergo self-specification prior to the formation of peripheral connections, and the outgrowing axons, unlike those in the amphibian spinal system, take special courses to terminate in specific premuscle masses. Evolutionary refinement in the developmental process may verge in the higher vertebrates toward a similar arrangement in the spinal system as well. Motor axons added after establishment of the pioneer connections might follow previously specified fibers to specific muscles. These, however, remain matters for further investigation.

Tectobulbar and Tectospinal Linkages

The foregoing has not ruled out the possibility that the adjustment of synaptic relations deeper within the centers, i.e. among the second- and higher-order neurons, might be subject to a rather different sort of regulation. Independence of the learning factor, at least, is indicated in data mentioned above on the development of visual reflexes following prefunctional rotation of the eyes, development of vestibulo-ocular responses in the absence of the optic nerve, development of limb coordination following prefunctional transplantation of the limb buds, and also in studies on the development of the swimming pattern under anesthesia (Carmichael, 1926; Harrison, 1904; Matthews and Detwiler, 1926). This kind of evidence, however, fails to eliminate the possibility that mechanical relations, along with a scheduled timing of neuron proliferation, differentiation, fiber outgrowth, and related phenomena, might be sufficient in themselves for the development of central organization, with no need for neuronal specification.

That the second-order neurons are, in fact, biochemically differentiated has been inferred already from the manner in which the primary sensory and motor synapses are formed. More direct evidence of the existence of qualitative specification among the association cells, and of its regulative role in the establishment of their synaptic associations, has been found in experiments dealing with regeneration of the secondary neurons of the visual system (Sperry, 1948*a*). From the primary visual centers of the brain into which the optic fibers discharge, second- and higher-order neurons running mainly in the tectobulbar and tectospinal tracts transmit the impulses of vision to the motor systems of the bulb and spinal cord. The efferent synaptic relations that these higher-order neurons maintain with the bulbar and spinal systems must be selectively adjusted to suit the differential afferent relations of the cell bodies and dendrites in thalamus and midbrain. For the

attainment of proper function in development or in regeneration, the efferent synapses have to be established in a discriminative manner. Otherwise distortion and confusion would appear in the visuomotor coordinations.

When the brain of the adult water newt, *Triturus viridescens*, is transected posterior to the optic lobes, somewhat rostral to the entrance of cranial nerve V and close to the level of the trochlear nuclei (Fig. 18), the tectobulbar and tectospinal tracts, plus the many other descending and ascending tracts passing through this level, are interrupted, with consequent functional defects including abolishment of all visual responses. In addition to being blind and anosmic, the animals are weak and deficient in purposeful direction of activity, and they also show abnormalities of posture, equilibrium, and coordination. Animals that manage to survive begin to show functional improvement during the third week. Optokinetic responses begin to reappear at approximately the end of the third week, and the ability to locate accurately small moving objects in space returns during the following weeks. At the end of approximately two months all the lost functions seem to have been restored in good order.

More than a dozen main fiber tracts are recognizable histologically at the level of transection (Herrick, 1936), and each of these has its own intrafascicular fiber differentiation. With conditions favoring an extreme jumbling and intermixing of fiber types in regeneration, the fact that the functional relations are nevertheless restored in orderly fashion points to the presence of specific regulative agents.

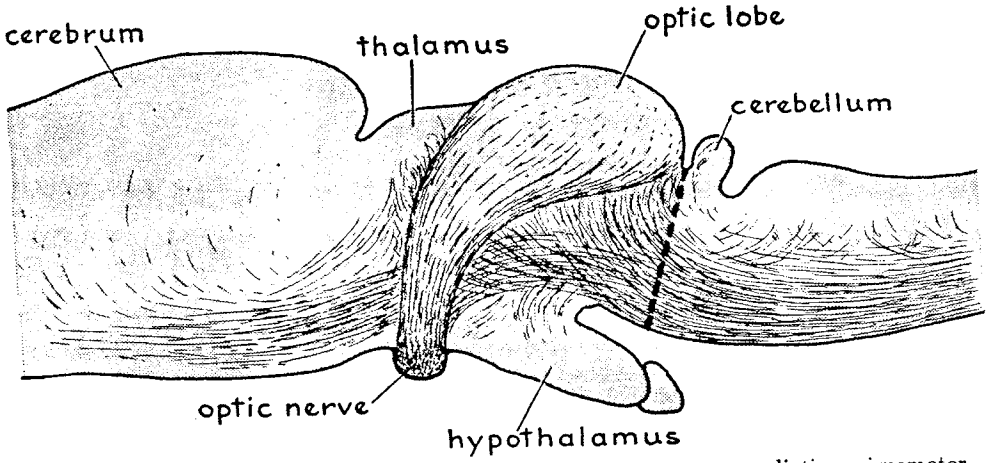

FIG. 18. Level of transection of the secondary central tracts mediating visuomotor coordination (from Sperry, 1948*a*). After transection of all the ascending and descending tracts that link the higher centers with spinal cord and medulla, regeneration leads to a systematic recovery of visuomotor and other coordinations, even though the eyes meanwhile have been rotated 180 degrees.

That the orderliness of recovery is not due to functional adaptation was shown by rotating the eyes on the optic axis 180 degrees in some of the animals during the period when the central tracts were regenerating. In such animals the visual responses upon recovery showed a systematic reversal in direction just like that produced by rotation of the eyes in animals otherwise normal.

With other possibilities eliminated we are forced to conclude that these higher-order

neurons, like the primary neurons, differ in character and form their synaptic associations selectively on the basis of constitutional specificities. Functional adaptation was not eliminated as a regulative factor in recovery of functions other than vision, but there is no reason for supposing that the other central fiber tracts that were transected reestablished their synaptic associations in a manner entirely different from those subserving vision. Good functional recovery has also been reported to follow regeneration of the transected spinal cord in amphibian larvae (Lorente de Nó, 1921; and others) and in certain teleosts (Hooker, 1932; Tuge and Hanzawa, 1937). Although adaptation through function has not been ruled out in these cases, it appears likely that here also the adaptation mechanisms are developmental, not functional, in character.

Patterning of Complete Reflex Circuits

From the data relating to the separate systems of sensory, motor, and central neurons, it is possible to assemble a tentative working picture of the developmental organization of the entire sensori-neuro-motor pathway of a few reflex circuits. The myotatic reflexes are mediated by a "two-neuron arc" consisting of the proprioceptive and the primary motor neurons (Lloyd, 1946). Evidence regarding the acquisition of specificity and central connections has been presented for both these sets of neurons, and we need add only the temporal order of events. The motor cells first form their peripheral connections and then develop wide-spreading dendrites. The afferent neurons meanwhile attain their end-organ relations and send their central root fibers into the cord to form ascending and descending branches. Later these send off numerous collaterals into the neuropil, some of which contact directly the dendrites and cell bodies of the motor neurons. In forming synapses the afferent fibers of each muscle apparently exhibit a special predilection for those motor neurons specified by the same muscle, and to a lesser extent for those of the most closely related muscles. This would seem to involve a simple affinity of like for like. Such simplicity in interneuron affinity is exceptional, however, and even in this case the same proprioceptive root fibers probably form connections on a different basis at other levels of the cord. Inhibitory associations also must be formed with antagonist motor neurons. Little can be said about the latter, however, in the absence of more information regarding the neural basis of reciprocal inhibition.

The cutaneous reflexes for withdrawal from irritating objects are more complicated, requiring at least one set of intracentral neurons (Lloyd, 1946). The number of distinct motor patterns employed in the localizing reflexes of the frog hind limb, for example, would require several specific classes of association neurons, each tending to activate a distinct combination of motor neurons. Specification of these association neurons must occur principally by self-differentiation. According to the usual order of development, the various classes of association cells probably form their selective relations with the motor neurons before the sensory connections are established. Neurons connected with different cutaneous regions presumably show a preference for specific types of association cells. The extent of functional variability would indicate that the neuronal affinities are not very strict and permit extensive overlap among the afferent terminations. Additional inhibitory associations must also be established among the internuncial cells so that the ascendency of one excitation pattern automatically inhibits all others.

The vestibulo-ocular reflexes for compensatory eye movements are mediated by a "three-neuron arc." Specification and synapsis of the sensory and motor neurons have already been discussed, and the existence of specificity among the association neurons located in the vestibular nuclei has been in-

ferred. The latter could be achieved by self-differentiation or, in part, through inductive effects from contact with the already differentiated motor ocular nuclei. In the former case the outgrowing axons from the vestibular nuclei would have to find their appropriate neurons in nuclei III, IV, and VI. Alternatively the association neurons might first establish their efferent relations in a more random fashion, with the final specification being induced by the motor nuclei.

Studies on the optic nerve, the secondary visual tracts, and the primary motor cells cover the main orders of neurons involved in optokinetic and other visual reflexes. Here also the developmental steps involve specification of the motor and sensory fibers under the influence of their end organs, plus a correlated but largely independent specification of the intracentral neurons. Connections are formed in the usual order first with the motor periphery, then between the central and motor cells, and finally the circuits are closed by ingrowth of the optic fibers. Without repetition of details, the evidence indicates that the synaptic junctions are formed in each case in a selective manner on the basis of interneuronal affinities. With regard to these as well as to the foregoing reflexes, only the most direct pathways have been considered. Alternative reflex routes often exist, and, according to Lorente de Nó (1933), the main reflex pathways have superimposed upon them local open and closed circuits. It is not difficult to visualize in a general way the serial organization of such accessory circuits through the subsequent spread of inductive effects after the main lines of differentiation have been laid down.

Not only the structural linkages but also the excitatory thresholds of the nerve cells must be properly adjusted. Little is known about the mechanisms controlling the latter. Apparently the different types of neurons and neuron groups acquire a characteristic resting threshold in the course of their differentiation. Shock phenomena suggest that the excitatory thresholds must be adjusted in development to the number and type of synaptic endings present. Cutting off some of the afferent connections of a neuron raises its excitatory threshold. In the course of time the neuron then tends to regain its characteristic threshold in new equilibrium with whatever afferent influences remain. Extrinsic trophic as well as nerve-discharge effects from the connecting cells thus seem to be involved, along with intrinsic metabolic processes, in determining the neuron's basal excitatory state.

It is evident that the central organization even of the relatively simple reflex patterns can be discussed as yet only in a simplified and speculative manner. The few inquiries we have thus far made into the complex picture of the organization of central synaptic relations reveal something of the possible nature of the mechanisms involved, but they only begin to suggest the complications of actual detail.

OTHER PATTERNING FACTORS AND CERTAIN THEORETICAL CONCEPTS

Neurobiotaxis

The theory most widely supported as an explanation of the structuring of integrative patterns is Kappers' (1932) law of "neurobiotaxis." Electrical currents are presumed in this theory to play a dominant role in determining neuron relations. It is maintained (1) that the neuron is electrically polarized, (2) that axons grow out from the cell body along the direction of an electric current and dendrites against the current, (3) that the nerve cell bodies tend to migrate along with dendritic growth against the current, (4) that dendrites and axons tend to grow perpendicularly toward and away from a neighboring fiber tract, owing to the electrical field set up around the tract by the passage of impulses, and (5) that the collection of similar fibers into bundles and the selective

linkage of particular neurons are due to the timing of electrical discharges in the developing nervous system. A related hypothesis proposed by Bok (1915) under the term "stimulogenous fibrillation" likewise assumed that outgrowing nerve fibers are directed by electrical irradiation from stimulated fiber tracts and also from sensory surfaces and muscles. The present discussion concerns only those aspects related to fiber outgrowth and synaptic formation.

The cogent criticisms of Weiss (1941*c*) cast strong doubt on the whole idea that electrical fields can play any major role in patterning the neuronal relations of the developing embryo. There are numerous phenomena in nervous development and regeneration, as, for example, the simultaneous growth of fibers in the same locality in different directions (Speidel, 1933), that are incompatible with the contention that the direction of fiber growth is electrically determined. Even some of the original observations on which the electrical theories were largely based have now been discredited (Hamburger, 1946; Windle, 1933). The report of a weak influence of electric current on nerve growth in vitro (Marsh and Beams, 1946) leaves open the possibility that certain aspects of the theory of neurobiotaxis may yet find verification. These, however, seem to have very limited explanatory value with reference to the patterning of functional interconnections.

Kappers himself was well aware that the postulated stimulo-concurrent character of axons and stimulopetal character of dendrites was not sufficient, per se, even in theory to account for the selective formation of interneuronal connections. To explain selective synapses he had to invoke the additional factor of timing. It is the differential timing of discharges in the developing nervous system that is supposed to cause selective linkage of particular neuron combinations. Connections form between neurons that happen to be excited simultaneously or in immediate succession. Thus the selective timing of neuron discharge is reasoned in circular fashion to cause the growth of selective structural relations on which is supposed to depend the selective timing of neuron discharge.

Any attempt to apply such a scheme to concrete cases leads to obvious and insurmountable difficulties. In the case of the retino-central connections it would be necessary to suppose that every animal of a species encounters exactly the same temporal and spatial pattern of retinal stimulation in the course of its development. Furthermore the light stimuli in each individual would have to be met in a schedule adjusted to the growth of the central dendrites with a precision utterly beyond the bounds of probability. Finally there is good evidence that the orderly projection of the retina upon the brain centers will develop even in complete darkness (Goodman, 1932; Hebb, 1937).

Recourse at this point to spontaneous retinal discharge is of no help, because the equally precise and systematic timing of the implicit discharges would then have to be accounted for. Assuming either implicit or overt discharges to be formative factors in the foregoing manner, it becomes necessary to suppose further that, once the various neurons have established their connections, they cease to discharge thereafter; otherwise they would continue to attract the growing fiber tips of all neurons developing subsequently, the terminals of which should be formed elsewhere. These and other difficulties discourage any attempt to make the concept of neurobiotaxis a primary formative factor in the ontogenetic patterning of synaptic associations. That something akin to neurobiotaxis may be involved in the learning process of the developed nervous system cannot be denied at this time in the face of our ignorance of the neural basis of learning. The speculative nature of any such contention should, however, be recognized.

Disuse Atrophy and Trophic Interdependence

The outgrowth of an excessive number of nerve fibers in development and in regeneration followed by atrophy and degeneration of those that fail to acquire terminal connections has been described by Cajal (1928, 1929) and others. The destruction of unconnected neurons and fiber branches is much slower in peripheral nerves than in the centers and is said to require months and perhaps years in the adult mammal. In the centers the process may occur within a few days. This phenomenon has been referred to by Cajal as the "law of utilitarian atrophy" or "atrophy of disuse."

That it is the "use" or discharge of the fibers that determines their survival, as might be inferred from the name, appears unlikely. In the first place the impulses of an axon, so far as is known, are conducted over those collaterals that are not terminally connected, as well as over those that are. Furthermore there are a number of instances in which the survival of neurons and end organs is known to depend upon the existence of adequate connections under conditions where the passage of excitations is clearly not the essential factor. For example, the degeneration of taste buds and of other sensory endings after severance of their nerve supply, the retrograde degeneration of motor horn cells in young mammals after destruction of their peripheral connections, and the retrograde degeneration of thalamic neurons after cortical lesions can hardly be attributed to lack of excitation, because ample sources of excitation under these conditions remain undisturbed. The functional relations are reversed in so-called "transneuronal" effects such as degeneration of the lateral geniculate nucleus after section of the optic nerve, degeneration in the red nucleus after section of the brachium conjunctivum, and degeneration of striated muscles after section of their somatic motor nerves. With regard to such phenomena also, however, there is evidence that the primary cause of degeneration is not the lack of excitation.

The responsible factor seems to be some kind of trophic, metabolic, chemical, or physical influence of these connections upon the nerve cells and their end organs. Apparently there is a general tendency for the elements of the sensori-neuro-motor system to become dependent in varying degree upon the linkages that they normally acquire. Accordingly some term such as "trophic degeneration" or "isolation atrophy" would seem more suitable for this class of phenomena.

To some extent the trophic interdependencies of neurons and end organs may be selective in character, as where motor fibers (Arey, 1941) or the sensory fibers of general sensibility fail to maintain taste buds in the tongue of mammals, or to maintain either taste buds or lateral-line organs in the skin of the catfish (Bailey, 1937). In other situations, however, there is considerable leeway in this respect, as where the same sensory fibers in the duck have been shown by skin transplantation to be capable of maintaining either the corpuscles of Gandry or the corpuscles of Herbst (Dijkstra, 1933).

In addition to the various phenomena of retrograde and of transneuronal chromatolysis, atrophy, and degeneration, there are certain other indications that linkages have a profound influence upon the general status of the neuron. The formation of terminal connections appears to act as an impetus to the differentiation of the embryonic neurons (Hamburger and Keefe, 1944). Terminal connections are also important in a selective manner for the attainment of normal fiber diameter (Aitken, Sharman, and Young, 1947; Weiss, Edds, and Cavanaugh, 1945). Acquisition of connections tends to bring about a state of equilibrium in the neuron, with cessation of further growth. Growing fibers are inclined to adhere to and to follow in a preferential manner other fibers that have succeeded in forming connections (Weiss, 1941c) as if there occurred a change in the surface of the connected fibers that distinguishes them from fibers still unconnected. Perhaps most important from the

point of view of the present discussion is the evidence already elaborated that the terminal cells and tissues may induce specification of neurons and thereby influence their central synapsis.

The fact that any fiber branches at all should remain unconnected in the organism deserves comment because, no matter where excess fibers may stray, there are always cells of some sort in the vicinity with which connections might be established as far as mechanical accessibility is concerned. That connections are, nevertheless, not always made indicates in itself a degree of chemical selectivity in neuron termination. The saturation and denervation effects discussed below are closely related phenomena.

It is conceivable that some kind of elimination of excess and maladaptive fiber connections is achieved on an essentially functional rather than on a trophic basis in the learning process in the developed nervous system. Unlike the foregoing growth and trophic phenomena, however, this has no direct observational foundation.

"Individuation versus Integration"

Influenced by certain Gestalt concepts in vogue at the time, Coghill (1929, 1930*a*) emphasized features of the development of behavior patterns that seemed to illustrate the application of Gestalt principles in this field. From his classical studies on the development of the nervous system and behavior in urodele larvae Coghill inferred that discrete responses always emerge out of larger patterns by a process of individuation. The organization of the larger, "total" behavior patterns he considered to be primary, not a secondary effect arising through integration of partial patterns. In studies of embryonic and fetal behavior much concern has been given ever since to the confirmation or refutation of this idea. Windle (1944) accepts Coghill's conclusions for salamanders but not for mammals, in which he has found discrete reflex responses appearing very early, with no prior mass movement or total pattern stage.

Coghill's conclusions in this regard were drawn primarily from behavioral evidence, although he was able to support the concept by certain anatomical correlations. The latter, however, were based largely on the diffuse outgrowth of the peculiar primary motor system of *Amblystoma*, which can hardly be considered typical of the organization of behavior patterns for higher vertebrates or even for succeeding developmental stages of *Amblystoma*. Precise anatomical correlates are not so easy to observe in higher vertebrates or in the later stages of urodele development, and accordingly most of the evidence from these sources is behavioral. What behavioral observations may mean regarding the nature of the underlying mechanisms is often difficult to infer. Individuation of behavior might conceivably be achieved in a variety of ways, as, for example, by the selective elimination or the addition of fiber collaterals, by the elimination or addition of entire neurons, by the selective reinforcement or the weakening of synapses already present, by the selective adjustment of excitatory thresholds, by the selective sensitization of neurons to each other, etc. On the other hand, the specificity of initial reactions need not necessarily imply absence of individuation from a "total pattern." It could mean merely that neural individuation is completed before the onset of function rather than after. Correlations between behavior and the developmental processes that produce the structure that mediates the behavior must frequently be rather remote.

When we consider the complicated developmental mechanisms involved in the patterning of interneuronal relations, "individuation versus integration" tends to fade as a crucial issue. As far as the evidence goes, there is nothing intrinsic to the nature of the developmental processes that would prevent the initial establishment of specialized, discrete functional relations. Also it is

clear that the development of "total" behavior patterns, particularly in the higher vertebrates, is dependent upon an array of developmental events occurring at all levels of the nervous system as well as in the periphery, many of which are widely separated and regulated quite independently of one another.

Myelinization and Function

The addition of myelin to nerve fibers probably has little or no direct influence upon the formation of proper reflex connections. It deserves mention, however, as a conspicuous feature in the development and maturation of the nervous system and as an indicator of the order in which various systems become functional. The central nervous tracts tend to become myelinated in a definite sequence that shows considerable constancy in all mammals and follows roughly the order in which the tracts were developed phylogenetically (Langworthy, 1933). A general correlation between the order of the appearance of stainable myelin and the order in which the nerves become functional has suggested a causal relation. The relation, however, is not simple, and it appears to be reciprocal in nature.

It has been shown by Langworthy that fairly complex activity can be carried out prior to myelinization. On the other hand, functions in general show considerable improvement in speed, strength, steadiness, and precision coincident with the laying down of myelin. However, it is not entirely a matter of myelinization acting to improve function, because, conversely, the laying down of myelin is stimulated and accelerated by function. At the same time absence of normal function does not prevent or decrease the ultimate degree of myelinization (Romanes, 1947). Myelinization and the order of its occurrence would seem thus to be an inherently predetermined process capable of proceeding in the absence of function in the usual sense. Function when present, however, stimulates and accelerates myelinization, which in turn appears to act reciprocally to improve function. It cannot be said to what extent the apparent myelinization-function interrelation is directly dependent upon these factors, per se, and to what extent upon other maturational processes that may be going on simultaneously.

Flux in Neuronal Associations

Studies of the details of nerve termination in the tail of the frog tadpole (Speidel, 1940–41), in the cutaneous plexus of the ear of the rabbit, and in human skin, particularly where it is subject to frequent stretching and abrasion (Weddell and Glees, 1942), support earlier contentions that the peripheral connections are not stable but are in a continuous state of flux with degenerative and regenerative changes commonly taking place among the terminal arborizations.

Speidel suggests that a similar state of flux probably characterizes the synaptic terminals in the centers.

With constant readjustment of this sort taking place, there must exist continuous opportunity for the establishment of atypical connections. In the skin, for example, there should be ample opportunity for temperature fibers to terminate in tactile "spots," and vice versa. There should be even greater chance for the formation of abnormal terminations in the closely packed central nuclei. That the general pattern of neuronal termination does not become severely disarranged by these changes means that there must be persistent influences acting to maintain the proper relations.

It is not just a matter of the regenerating fibers' being guided back along their previous pathways to the former terminals. Speidel (1940–41) states that the regenerated pattern distal to the neurilemma sheaths bears no specific resemblance to the original pattern. Weddell and Glees (1942) describe dichotomy in regenerating fibers indicative of new collaterals, and the sprouting of new collaterals into neighboring denervated areas is well authenticated (Wed-

dell, Guttmann, and Gutmann, 1941). Apparently the same type of selective force that determines the original pattern of connections in development continues to be effective in maintaining the general pattern after it is formed. The range of possible terminations of each neuron may be restricted by its constitution and by its affinities for the various cell types within its terminal area. This would permit some variability to occur in the terminations of individual fibers but would keep constant the overall pattern of termination.

Selective Fasciculation

During the outgrowth of fibers from spinal cord grafts into nearby grafted limbs (Weiss, 1941*c*) those pioneer fibers that first become attached to the limb acquire thereby some property that converts them into a preferential contact pathway for later fibers. Subsequent fibers adhere to earlier fibers that have made terminations in preference to fibers that have not. Axon fibers of supernumerary Mauthner's cells, the giant neuron of the fish brain, exhibit a marked tendency to follow the original Mauthner's fibers (Oppenheimer, 1942). Also, grafted ectopic cranial nerve roots tend to enter the brain at the same level as the corresponding host nerves to which they become adherent (Piatt, 1947). Experimental observations of this sort, plus the fact that nerve fibers of similar type tend normally to collect together in separate fascicles in the neuraxis and to some extent in the peripheral nerves, indicate that outgrowing nerve fibers adhere selectively to other fibers of their own kind, for which Weiss (1941*c*) has suggested the term "selective fasciculation."

As new fibers are added to the spinal nerves during growth, the outgrowing motor fibers apparently turn into muscular branches at each bifurcation, whereas cutaneous fibers avoid muscular branches and turn into cutaneous pathways (Taylor, 1944). Something must prevent the motor fibers from following cutaneous branches, and vice versa. Moreover both motor and sensory fibers distribute themselves in proper proportions to their respective peripheries. Something, therefore, must prevent the motor fibers from all turning into the same motor channels, leaving other branches undersized. A tendency for fibers to stick selectively to preceding pioneer fibers of their own kind would conceivably help to account for this proportionate distribution of new fibers.

Saturation and Denervation Effects on Fiber Termination

Under normal conditions nerve endings tend to be limited to a characteristic density, even in situations where it is clear that many more terminations could be established were it merely a matter of space and numbers of available fibers. In muscle, for example, the nerve terminations are distributed in a strictly limited manner to the individual muscle fibers and spindles. One may well ask why some muscle fibers do not get several dozen axon terminals, others half that many, and some none. Similarly in the skin the nerve endings tend to be scattered evenly. The same is true of some of the central nuclei. In the lateral geniculate nucleus, for example, the telodendria of the optic axons are distributed evenly, with a characteristic number of endings on each geniculate cell.

One of the reasons advanced to explain the fact that neurons and end organs do not become smothered with nerve terminals beyond a characteristic limit is that nerve connections cause a reaction in the cells of termination that makes these cells impervious to the advances of additional fibers (Harrison, 1910; Weiss, 1941*c*). When the saturation point, which varies for different cells and tissues, is reached, conditions become unfavorable for further fiber growth and termination. The situation has been compared to that of the fertilization of eggs in which the penetration of the first spermatozoon causes a reaction in the egg cell, pre-

venting the entrance of more sperm. Some experimental evidence for this has been found in the innervation of muscles and of whole limbs (Fort, 1940; Litwiller, 1938). The fact that some fibers in central tracts terminate abruptly in nearby centers while others push on to much more distant regions might be due in part to the successive saturation of the various areas. The additional possibility must be considered that, in the centers, neurons may reach a saturation point for certain types of synapses while still accepting others.

The opposite phenomenon has also been observed, namely, a stimulative effect of denervated areas on nerve fiber outgrowth (Speidel, 1940–41; Weddell, Guttmann, and Gutmann, 1941). Fort (1940) found that motor nerve fibers grow out faster in denervated muscle than in innervated muscle, and that, the longer the muscle is denervated, the faster the outgrowth within the time limits he investigated. Geohegan and Aidar (1942) report experiments suggesting that partial denervation of the sympathetic ganglia causes the growth within the ganglia of collaterals from the remaining intact fibers to supply the denervated cells. However, in this case it is difficult to be sure how much the effect may be due merely to hypersensitivity of the denervated neurons.

Central Self-Differentiation of Coordination Patterns

The central patterns of coordination develop with considerable independence of connections with the sensory periphery. In the early stages of differentiation the motor system, as a rule, achieves a precocious organization of its own so that spontaneous or central excitations lead to coordinated motor responses before any sensory connections have been established (Coghill, 1929; Herrick, 1939; Windle, 1944; and others). It has also been shown that coordinated limb movement develops after prefunctional excision of the sensory nerve supply of the limb itself (Weiss, 1941*d*; Taylor, 1944). To this extent and to the degree that developmental organization does not depend on experience, central patterning seems to be independent of peripheral influences.

The foregoing, along with data on the autonomous nature of functional organization in the centers has led in some instances to special emphasis on the extent to which the basic patterns of coordination undergo an autonomous central self-differentiation. However, the synaptic relations of the primary sensory and motor neurons form an important part of the central pattern. If formation of these primary synaptic associations is governed by inductive effects from the periphery, then our conception of the extent to which coordination patterns undergo central self-differentiation must be adjusted accordingly. It has not yet been possible to demonstrate the development of functional organization in the nerve centers in the absence of connections on the motor side. Thus the possibility that inductive effects from the motor endings play a role in regulating the formation of neuronal interconnections in the centers has not been excluded. Nor can it be said that the present evidence completely eliminates the possibility that the ingrowth of sensory root fibers into their various central nuclei has some influence on the differentiation therein of functional relations. It has been found, for instance, that aberrant VIII and lateral-line root fibers will induce the differentiation of supernumerary Mauthner's cells in atypical sites where they enter the brain (Piatt, 1947).

Even with such allowances, however, the role of central self-differentiation in the development of coordination patterns still deserves emphasis. The differentiation or specification of the secondary and especially of the higher-order neurons and the consequent patterns of interrelations that they form must depend principally on formative agents within the centers. Differences in the inherent behavior patterns of different vertebrate species from the level of complex in-

stincts down to that of polysynaptic reflex responses must for the most part be attributed to central and not to peripheral influences. In earlier discussions of the chemoaffinity hypothesis (Sperry, 1943*c*) the induction of central organization starting from the periphery and working inward has certainly been overemphasized.

In nuclear fields like the optic tectum or the striate cortex in which the functional differentiation is correlated with anatomical dimensions in a gradient, field fashion, a basic self-differentiation of the nucleus is indicated. There are many nuclei of this type that may, along with the peripheral structures, be looked upon as foci of primary self-differentiation. On the other hand, there are central nuclei, such as the spinal association centers and the reticular areas of the brain stem, in which neurons of similar functional type appear to be scattered in a more diffuse manner among other neurons of diverse functions. In such regions it is possible that specification is more dependent upon terminal contacts, as with the limb motor neurons. Neuron specification may thus arise from intrinsic self-differentiation or from extrinsic inductive actions. The latter may sometimes be effected over considerable distances through axon and dendrite contacts. Both types of specification are probably involved in varying degree in many nuclei.

Functional Reinforcement of Adaptive Patterns

It has been a common conception that synaptic linkages are adjusted in development on the basis of functional adaptiveness. Usually it has been supposed that synaptic associations are laid down initially in a diffuse, excessive, and fortuitous fashion, after which there occurs a selective reinforcement of the adaptive linkages with atrophy and degeneration of the maladaptive linkages. In the learning process in the mature animal, some kind of selective retention on the basis of functional effects does take place, of course, and it has been an easy step to infer its occurrence in development also.

In most vertebrates, however, up to and including the lower mammals (Sperry, 1945*c*), the learning capacity, after it has reached its peak in the adult, is still unable to effect any rearrangement of the basic integrative structure of the nervous system. Even in the primates learning appears to be largely cerebral. It effects no changes in the interneuronal relations of cord and brain stem (Sperry, 1947*c*). To assume that the elementary integrative relations are organized in the first place through function is thus to assume that the nervous system in its imperfect, immature condition, and under circumstances unfavorable in many respects for functional adjustment, is able to handle a type of learning of which it is incapable later on.

It is conceivable, in this regard, that neural tissue might be more plastic in the developmental stages. Learning and growth together might, through a series of irreversible steps, produce integrative structures that, when completed, would no longer be subject to functional reorganization. However, the learning capacity of the nervous system is much more than a mere passive plasticity of a highly impressionable tissue. It is more comparable to the active functional ability of a complex machine. The self-regulative, operational organization of the nervous system, not just the character of its protoplasm, is what enables it to utilize its experience selectively to improve upon its own structure. Complex self-regulative mechanisms must first be constructed in ontogeny before adaptation by learning can begin to take place.

The selectivity of learning is difficult to account for in early development. The fact that not all excitation patterns, but only the adaptive ones, reinforce themselves must be explained. Some background of organization must be present for different excitation patterns to have different reinforcement value. Otherwise all reactions would

tend to reinforce themselves indiscriminately. The establishment of a central organization capable of distinguishing adaptive from nonadaptive excitation patterns must therefore logically precede any selective adjustment on the basis of functional effects.

The point in nervous development at which sufficient organization is attained to permit selective reinforcement on the basis of functional adequacy might conceivably be an early one, but the evidence indicates not. In mammals it is doubtful if any appreciable patterning of synaptic associations is achieved by function before the cerebral cortex has become differentiated and has established its subcortical connections. As this is one of the last steps of nervous development, it may be concluded that the bulk of the nervous system must be patterned without the aid of functional adjustment. From the developmental and anatomical point of view, the synaptic modifications that are later imposed by the learning process constitute a rather minute refinement in the nervous structure, so inconspicuous, in fact, that the exact location and even the nature of these learned adjustments still await demonstration.

The foregoing concerns function as a patterning factor in the organization of neuronal connections and does not apply to function as a general condition necessary to healthy growth. Development in many instances, however, is remarkably independent of function, even in this latter sense. The experiments of Harrison (1904) and others demonstrate the orderly formation of the amphibian nervous system well through the swimming stage under deep anesthesia. It is the positive toxic effects of the anesthesia, presumably, rather than the absence of function per se that prevents development from proceeding much farther under these conditions. Rabbits reared to the age of six months in complete darkness have been found to have neither gross nor histological defects detectable anywhere in the visual system, including the cortical projection area (Goodman, 1932). A general tendency is evident in other organ systems, as well as in the nervous system, for development to be independent of function and to anticipate the needs of function. But function sometimes becomes a necessary element later on, after it has once started.

The stage at which function becomes a formative factor and its relative importance in neurogenesis obviously varies greatly in different species. Function plays its paramount role in the postnatal development of man. Where for a long period both function and maturation are operative together, the contributions of each to the final structure become interrelated in complex manner. In attempting to interpret the defective development of vision in chimpanzees reared in the dark from birth (Riesen, 1947), for example, it is extremely difficult to untangle the relative roles played by the three different factors: inherent maturation, function as a specific organizing agent, and function as a nonformative but necessary condition. One would hardly expect the lack of light in these cases to cause disorderly development of the cortical projection of the retinal field. However, retinal degeneration and inability to respond normally to light for almost a month have been observed in fish kept in the dark for more than two years (Breder and Rasquin, 1947; Ogneff, 1911). In certain cave salamanders, the larval eyes of which normally undergo regression at metamorphosis, the eyes will become quite normal if the larvae grow to adulthood in the light (Walls, 1942).

Finally it is usually impossible to assess the relative importance of maturation and of learning with reference to behavior, because most behavior depends upon a combination of neural structures, of which some may be inherently organized and others organized by experience. The issues are considerably clarified by restricting the problem to the organization of the neural structure itself. In this case the patterning of certain parts can be ascribed entirely to inherent growth

forces, whereas in others the learning factor may be involved in varying degree.

Hormones and Maturation

In infraprimate mammals and especially in lower vertebrates it is evident that many of the reactions associated with reproduction, such as nest building, mating, and giving birth to, feeding, and caring for the young are largely inherent in character. The maturation of these particular reaction patterns, some of them extremely elaborate, is of especial interest because they appear so late in life and because their appearance is correlated with the perfusion of the nervous system with specific chemical agents. Similarly in the metamorphosis of anuran amphibians there occurs a relatively late maturation of a whole series of new behavior patterns dependent in part, at least, upon hormonal action.

A precocious localized maturation of the lid-closure reflex has been induced in frog tadpoles (Kollros, 1942) by implanting thyroid pellets into the hindbrain. Other lines of evidence suggest that the reproductive hormones likewise may act directly and selectively upon the nerve centers (Beach, 1947). There is also considerable specificity in the behavioral effects of the male and female hormones. This selective response of particular neural mechanisms to biochemical agents represents another indication of chemical differentiation within the centers. To it may be added a host of related phenomena involving selective susceptibility of different neuron types to the action of various drugs, toxins, histological stains, metals, viruses, bacteria, etc.

The exact manner in which hormones act on the centers is not known. It is fairly certain with regard to the sex reactions of many mammals, at least, that the patterns of nerve cells involved are well differentiated and their functional relations basically arranged early in development, long before the increased concentration of hormone brings the mechanism into functional maturity (Beach, 1947). It is usually assumed that the hormone influences the excitation threshold of specific patterns of neurons, the structural relations of which remain fixed. However, in view of Speidel's (1940–41) suggestions regarding the lability of central associations, and in view of the duration of the latent period between the onset of hormone administration and its effect on behavior, it is possible that growth and readjustment of synaptic relations may also be involved.

Neurogenesis and Specific Nerve Energies

The experiments on synaptic development and regeneration have here been interpreted entirely on a connectionist basis. This discussion has been presented in terms of the classical assumption that the integrative action of the nervous system is dependent upon refined selectivity among neuronal linkages. However, there has arisen of late a good deal of skepticism concerning this traditional connectionist theory (Bethe, 1931; Gerard, 1941; Goldstein, 1939; Koffka, 1935; Köhler, 1940; Lashley, 1942; Weiss, 1936, 1941*d*, etc.). Accordingly it should be stressed that there is as yet no final proof that neuron specificities determine the growth of anatomical relations. In regeneration of the optic nerve, for example, it has not been directly observed that fibers from different retinal quadrants form different central connections. This is still inferential, and at least one other interpretation is theoretically possible; i.e. it is conceivable that the central connections are laid down in a profuse and random fashion and that the subsequent specificity of response derives from some sort of specificity in the nature of the impulses carried by the different fiber types.

At the turn of the century Hering (1913) argued in favor of the existence of qualitative specificity among the excitatory energies conducted by different types of nerve fibers. It was his contention that the pattern of the central pathways excited was determined not merely by the existing anatomical connec-

tions and their threshold phenomena but also by selective sensitivity of central neurons to specific types of afferent energies. He believed that the individual nerve impulses differed intrinsically in their physicochemical properties. As an alternative he mentioned that the specificity might also reside in frequency differences, in which case the specific energies of the nerve fibers would appear as special resonance capacities and the central patterns would be determined by selective attunement of the various neuron types to different frequencies (see also Head, 1920). Later, similar concepts were invoked (Weiss, 1928, 1936, 1941*d*) as a possible explanation of homologous response in supernumerary amphibian limbs. The "homologous" or "myotypic" action of muscles atypically innervated seemed to rule out specific anatomical connections on the part of the motor neurons and to indicate instead a selective physiological sensitivity to specific excitatory agents, one for each muscle, within the limb centers.

All our experiments on the formation of synaptic associations could be interpreted on a similar physiological basis rather than in terms of the growth of specific anatomical connections. Discussion of this alternative possibility has been purposely postponed until now in order to avoid confusion and repetition. Once the connectionist explanation is clear, it becomes a relatively easy step — should future findings necessitate it — to translate the results and conclusions into terms of specific nerve energy or resonance phenomena. The connectionist interpretation is emphasized at present only because the great mass of neurological data fall more readily into this traditional scheme. Furthermore, with a connectionist explanation of homologous response now available, the original reasons for resorting to a resonance hypothesis have been largely removed.

For the sake of completeness mention should be made of another possible basis of interpretation. It is conceivable (Sperry, 1943*c*) that neurons might regularly form and maintain an excess of connections, but that only a selective minority of the synapses ever become functional. The interneuronal affinities might affect the structure of the synapses to make certain ones capable of transmitting excitations and others not. Functional specificity would still depend on selective anatomical associations rather than on resonance phenomena or specific nerve energies, but only minute differences in synaptic structure would be involved instead of complete presence or absence of synapses.

SUMMARY

A survey of the evidence supports the thesis that the inherent patterning of neuronal linkages is achieved by embryonic processes similar to those responsible for the grosser phases of neurogenesis and for the development of other organ systems. Among these developmental processes those of embryonic differentiation, determination, and induction deserve special mention. In the genetic structuring of the integrative circuits developmental forces reach their peak of refinement and complexity.

Just as the cells of the early embryo differentiate into a variety of strains to form the diverse tissues of the adult body, so the cells of the nervous system undergo a similar, though more subtle, differentiation among themselves into a multitude of neuron types. Not only the nervous system but the associated peripheral tissues as well, the musculature and its tendons, the dermis, the fascias, the ligaments, the periosteum, the retina, the cochlea, etc., are subject to a refined qualitative differentiation or specification far beyond what is visibly manifest.

After the prospective nervous tissue has become differentiated from other tissues and has formed the neural tube, the basal or motor portion of the tube differentiates from the sensory or alar portion and the gross cephalic regions become distinct — this and the following by way of illustration and without regard for the exact order of these

events. An eye field emerges in the diencephalic region, and in the spinal cord a brachial and a hind-limb field are differentiated. The main subdivisions of the brain begin to take form, and more localized foci of differentiation subdivide these into their major nuclear fields. By transplantation methods it can be demonstrated that these various local fields differ from each other in their constitutional properties.

Differentiation continues until in many nuclei it approaches the level of the individual nerve cell. For example, in the optic field of the midbrain the cells in any given locus differ from those in all other loci. The nearer together the neurons, the more alike they remain in constitution, but even neighboring cells may differ to some extent, depending upon the steepness of the differentiation gradient across the nucleus. The development of Mauthner's neuron in the medulla illustrates an extreme example in which a single nerve cell consistently differentiates in visible manner from among its fellows to acquire specific structural and functional characteristics.

This refined specification of the neurons makes possible the formation of selective synaptic linkages on the basis of a chemoaffinity. By virtue of their specification the advancing tips of growing nerve fibers form synapses only with particular ones of the various kinds of neurons they encounter in their outgrowth. The formation of precisely designed integrative circuits thus becomes possible without the aid of functional adjustment. Synapses may be neatly arranged even in regions that appear under the microscope to be only disorderly tangles of axons, dendrites, and cell bodies. The chemical interrelations of the nerve cells must be extremely complicated. This is doubly true where axons maintain inhibitory linkages in addition to their excitatory synapses. According to the chemoaffinity principle a neuron could not maintain dissimilar axon linkages on the two sides of the midsagittal plane, in the absence of a left-right specification of corresponding cells on each side. In the few examples studied, including neurons of the optic tract, the tectobulbar tract, and the dorsal roots, the fibers seem to form with equal readiness the same pattern of synaptic associations on either side of the midline. A left-right selectivity, especially among the commissural neurons, however, has not been excluded and is not inconceivable.

The induction of cell differentiation through contact with other cells and tissues, common throughout development, takes on a special aspect in the nervous system. Owing to the tremendous elongation of the neuron processes, induction effects may occur over long distances between widely separated cells. It becomes possible for a group of neurons to be similarly specified through similar termination even though their cell bodies are scattered diffusely among other neurons of diverse character. Neuronal differentiation thus tends to be released from some of the limitations of topography that apply to the development of other organs. Much greater diversity and refinement is possible than where induction depends upon immediate contact of the cell bodies themselves.

It has been emphasized that these qualitative specificities operate in conjunction with many other factors, and the combined action of all of them determines the final pattern. The unquestioned importance of stereotropism and of topographic and chronological relations must be taken for granted throughout. Certain other formative factors such as trophic dependencies, saturation and denervation effects, excitatory threshold adjustment, etc., are also involved, but as yet they are little understood.

Some of the salient features in the patterning of certain elementary integrative circuits are considered as far as the experimental evidence permits. These concrete examples illustrate the manner in which developmental mechanisms may operate without the aid of function to insure precise

neuronal connections. Proper peripheral-central linkages, for example, are assured in many instances by having the central connections dependent upon specificity induced in the nerve fibers by their end organs.

Judging from the variation in other aspects of development among different species of vertebrates, we would hardly expect the details of these processes, as observed in the amphibians, to be maintained throughout the vertebrate series. The general plan of development and the general nature of the maturational mechanisms involved, however, presumably are not subject to any abrupt or radical modification in phylogeny.

The foregoing has been concerned almost exclusively with the development and maturation of the integrative *structure* of the nervous system. The broader problem of the maturation of *behavior* with its corollary problems regarding the relation between neural structure and function have been avoided.

Our repeated reference to the role of anatomical connections in shaping response patterns need not lead to an exaggeration of the importance of this factor relative to others. Dependence of orderly function upon the neural architecture by no means excludes dependence upon other factors. The fluctuating patterns of facilitation and inhibition, which are continually playing through the anatomical structure, constantly condition in varying degree the character of the response. Particularly with respect to the temporal organization of behavior over increasing periods of time, the regulative role of these dynamic factors rapidly increases in interest and importance. Caution is also necessary with regard to implications concerning the nature and specificity of synaptic connections. For the most part it is possible merely to infer that the qualitative specification of neurons is closely correlated with differences in their synaptic associations. Little can be said concerning the exact nature of these synaptic differences.

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